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(57) Abstract

The present invention provides an isolated protein obtainable from a plant source which has antifungal activity, specifically anti–*Phytophthora* activity and/or anti–*Pythium* activity and a molecular weight of about 55–65 kDa as judged by SDS PAGE–electrophoresis, an isolated DNA sequence comprising an open reading frame capable of encoding a protein according to the invention, preferably characterised in that it comprises an open reading frame which is capable of encoding a protein depicted in SEQ ID NO. 16, SEQ ID NO. 57, SEQ ID NO. 70, SEQ ID NO. 72 or SEQ ID NO. 74 or muteins thereof, and DNA capable of hybridising therewith under stringent conditions. The invention further comprises plants incorporating chimeric DNA capable of encoding a protein according to the invention, and wherein the protein is expressed. Also shown is the carbohydrate and preferably hexose oxidating activity of said protein. Also methods are provided for combating fungi, especially *Phytophthora* and *Pythium* species, using a protein or a host cell capable of producing the protein.

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ANTIFUNGAL PROTEINS, DNA CODING THEREFORE, AND HOSTS INCORPORATING SAME

FIELD OF THE INVENTION

The present invention relates to new oxidases, which can act as antifungal proteins, DNA coding therefor and hosts incorporating the DNA, as well as methods of combating fungal pathogens by causing said fungal pathogens to be contacted with said protein or proteins. The invention further relates to plants, incorporating and expressing DNA coding for antifungal proteins, and to plants which as a result thereof show reduced susceptibility to fungal pathogens.

BACKGROUND ART

Fungal diseases of crop plants have been one of the principal causes of crop losses throughout the history of crop cultivation. The growing of crops as monocultures encourages the proliferation of virulent races of fungal pathogens and wherever a new variety of crop plant becomes grown on a wide scale of the risks of a virulent strain of a pathogen evolving to attack that crop increase drastically. The occurrence of disease is significantly worsened by the international transport of pathogen-carrying plant materials, which can bring together plants with pathogens against which they have had no opportunity to evolve resistance. Thus by man's intervention the natural balance between host and pathogen has been disturbed with disastrous effect on a number of occasions. Catastrophic losses and even famines such as occurred in Ireland during the 19th century, caused by the potato blight fungus (*Phytophthora infestans*) have resulted from such activities. Fungal disease can also make it completely impossible to grow certain crops in large areas, as was the case when *Fusarium* wilt wiped out tomato growing in large areas of the Eastern USA or the downy mildew (*Plasmopara viticola*) fungus devastated vine growing in parts of Europe. Outbreaks of fungal disease can also have a severe effect on the environment as happened when almost the entire English Elm (*Ulmus procera*) population was destroyed by Dutch Elm Disease (*Ceratocystis ulmi*). In addition the losses which may be caused during the growing of crops fungal disease may contribute to further post harvest losses. Various soft rots such as *Botrytis cinerea* are particularly problematic in soft fruit, for

example. The fungus *Aspergillus flavus*, although not a true disease-causing fungus, causes post-harvest rot on stored peanuts and maize, especially in tropical countries and is most serious because it produces a toxin, aflatoxin, which is very toxic to man.

The major economic problems associated with fungal diseases are found in wetter parts of the World, principally Western Europe and the humid tropics. Various crop husbandry techniques, such as crop rotation and avoiding the spread of soil on machinery etc., are used to prevent the build up and spread of severe infestations of fungal disease. Plant breeding has made a significant impact on improving the resistance of many crops to important diseases. For example, plant breeders successfully introduced resistance genes *1* and *1-2* effective against *Fusarium oxysporum* f.sp. *lycopersici* into tomato. Nonetheless, problems remain, particularly when many forms of race-specific resistance break down as new races of the pathogen rapidly evolve. In tomato, another virulent strain of *F. oxysporum* has occurred and breeders are seeking a third useful resistance gene. In cereals growing in parts of Western Europe a recent outbreak of a virulent strain of yellow rust (*Puccinia striiformis*) has lead to a rapid increase in fungicide use on varieties which remain resistant to other fungi. In these specific cases chemicals are widely used to control fungal disease, as in cases where there are simply no natural sources of resistance available to the breeder.

Chemical fungicides remain a major input in the costs of crop production in many parts of the World. In 1990 21% of all agrochemical sales were accounted for by fungicides (US $ 5,54 million). Farmers and growers have a strong motivation to reduce their input costs. Added to the economic justification is an increasingly strong environmental component in the equation. There is growing pressure in the more advanced economies, notably in North America and Western Europe, from politicians and consumers for agriculture which relies less on chemical inputs. The justification for such demands may lack focused rationale or scientific proof, but fears grow with reports of pesticides traced in groundwater or detected in quantities exceeding the minimums acceptable as food residues. In The Netherlands, for example, there is a mandatory requirement to reduce total pesticides use by 50% before 2000.

Phytophthora infestans belongs to the group of fungi referred to as Oomycetes. *Phytophthora infestans* infects various members of Solanaceae, such as potato, tomato and some ornamentals. It causes late blight of potatoes and tomatoes affecting all parts except roots. Geographically, the fungus is widely distributed, and it can be found in all potato-producing countries. Economically late blight in potatoes is of major importance, as infection early in the season can severely reduce crop yield. Currently the disease is controlled by spraying chemical fungicides (dithiocarbamates, such as mancozeb, manec and zineb) regularly. Both from an environmental and economical point of view, biological control of diseases caused by *Phytophthora infestans* could have advantages over the use of chemical fungicides.

Pythium also belongs to the group of fungi referred to as Oomycetes. The genus *Pythium* differs from the related genus *Phytophthora* by forming relatively undifferentiated sporangia. Geographically, this fungus is widely distributed on all continents. The first main type of disease caused by *Pythium* species is damping-off, due to sudden and fast developing attacks on young seedlings in the field or in nurseries. *Pythium* species cause a second type of disease which is root necrosis and causes a general slowing of plant growth (for example wheat and maize) and loss of yield.
The main losses caused by *Pythium* in Europe are to field crops such as sugarbeet. In principle, losses tend to be all-or-nothing. Similarly, nursery sowings of ornamentals and forest trees may be completely destroyed. (For a review on Oomycetes, *vide*: European Handbook of Plant Diseases, ed. by I.M. Smith *et al.*, 1988, Blackwell Scientific Publications, Ch.8)

Another fungus is *Botrytis*, especially *B. cinerea*, belonging to the group of *Fungi Imperfecti*, which causes gray mold blight or bud and flower blight, which is common on soft ripe fruits after harvesting, but it can also occur before harvest. It can also affect various vegetables such as lettuce, beans and tomato. Other species of *Botrytis* are common on flowers, such as lilies, gladiolus and tulips.

A protein with antifungal activity, isolated from TMV-induced tobaccc leaves, which is capable of causing lysis of germinating spores and hyphal tips of *Phytophthora infestans* and which causes the hyphae to grow at a reduced rate, was disclosed in WO91/18984 A1. This protein has an apparent molecular weight of about 24 kDa and was named

AP24. Comparison of its complete amino acid sequence, as deduced from the nucleic acid sequence of the AP24 gene, with proteins known from databases revealed that the protein was an osmotin-like protein.

Despite initial success in combating fungal pathogens, such as *Phytophthora infestans*, and the genetic engineering of plants capable of producing these antifungal proteins with activity against this fungal pathogen there remains a need to identify and isolate other proteins with antifungal activity against this fungus.

SUMMARY OF THE INVENTION

The present invention provides an isolated protein obtainable from a plant source which has antifungal activity, most effectively directed to Oomycetes, and preferably to *Phytophthora* and/or *Pythium* and a molecular weight of about 55-65 kDa as judged by SDS PAGE-electrophoresis. Preferred proteins are those that are obtainable from sunflower or lettuce plants. Even more preferred proteins are obtainable from sunflower or lettuce leaves induced with sodium salicylate. A still more preferred isolated protein is characterised in that it is selected from the group of proteins having the amino acid sequence selected from the group comprising of the amino acid sequences depicted in SEQ ID NO's: 1, 2 or 6, 16, 20, 49, 50, 51, 58, 71, 73 or 75 as well as muteins thereof which have antifungal and especially anti-*Phytophthora* and/or anti-*Pythium* activity. A still further preferred protein according to the invention is one characterised in that it comprises a protein that comprises the amino acid sequence as represented by SEQ ID NO's: 16, 20, 58, 71, 73 or 75 or by a part of said sequence like represented in SEQ ID NO: 6.

The invention also provides a new enzyme, the enzymatic activity being oxidation of carbohydrates.

The invention also embraces an isolated DNA sequence comprising an open reading frame capable of encoding a protein according to the invention, preferably characterised in that the open reading frame is capable of encoding a protein according to the invention, and DNA capable of hybridising therewith under stringent conditions.

The invention also provides a chimeric DNA sequence according to the invention further comprising a transcriptional initiation region and, optionally, a transcriptional termination region, so linked to said open reading frame as to enable the DNA to be transcribed in a

living host cell when present therein, thereby producing RNA which comprises said open reading frame. A preferred chimeric DNA sequence according to the invention is one, wherein the RNA comprising said open reading frame is capable of being translated into protein in said host cell, when present therein, thereby producing said protein. Especially preferred are DNA sequences comprising a sequence as depicted in SEQ ID NO's: 15, 19, 57, 70, 72 or 74.

The invention also embraces a chimeric DNA sequence comprising a DNA sequence according to the invention, which may be selected from replicons, such as bacterial cloning plasmids and vectors, such as a bacterial expression vector, a (non-integrative) plant viral vector, a Ti-plasmid vector of *Agrobacterium*, such as a binary vector, and the like, as well as a host cell comprising a replicon or vector according to the invention, and which is capable of maintaining said replicon once present therein. Preferred according to that embodiment is a host cell which is a plant cell, said vector being a non-integrative viral vector.

The invention further provides a host cell stably incorporating in its genome a chimeric DNA sequence according to the invention, such as a plant cell, as well as multicellular hosts comprising such cells, or essentially consisting of such cells, such as plants. Especially preferred are plants characterised in that the chimeric DNA according to the invention is expressed in at least a number of the plant's cells causing the said antifungal protein to be produced therein.

According to yet another embodiment of the invention a method for producing a protein with carbohydrate oxidase activity is provided, characterised in that a host cell according to the invention is grown under conditions allowing the said protein to be produced by said host cell, optionally followed by the step of recovering the protein from the host cells.

Another part of the invention is directed to the antifungal use of a protein which has carbohydrate oxidase activity.

The invention provides also for the use of a protein according to the invention for retarding the growth of fungi, preferably Oomycetes and more preferably *Phytophthora* and *Pythium*. According to yet another embodiment, retarding the growth of the fungi is on or in the neighbourhood of the plant by applying a microorganism capable of producing the protein or by harvesting the protein from a microbial

host and applying the protein in an agrochemical formulation.

The invention also provides a method for obtaining plants with reduced susceptibility to fungi, especially *Phytophthora* and/or *Pythium*, comprising the steps of

(a) introducing into ancestor cells which are susceptible of regeneration into a whole plant,

- a chimeric DNA sequence comprising an open reading frame capable of encoding a protein according to claim 1, said open reading frame being operatively linked to a transcriptional and translational region and, optionally, a transcriptional termination region, allowing the said protein to be produced in a plant cell that is susceptible to infection by said fungus, and
- a chimeric DNA sequence capable of encoding a plant selectable marker allowing selection of transformed ancestor cells when said selectable marker is present therein, and

(b) regenerating said ancestor cells into plants under conditions favouring ancestor cells which have the said selectable marker, and

(c) identifying a plant which produces a protein according to claim 1, thereby reducing the susceptibility of said plant to infection by said fungus.

Preferred according to the invention is a method characterised in that step (a) is performed using an *Agrobacterium tumefaciens* strain capable of T-DNA transfer to plant cells and which harbours the said chimeric DNA cloned into binary vector pMOG800; another preferred method is when step (b) is performed in the presence of an antibiotic favouring cells which have a neomycin phosphotransferase.

The invention further provides an antifungal composition comprising a protein according to the invention and a suitable carrier.

An antibody, capable of reacting with an N-terminal fragment of a protein according to the invention, preferably to the peptide represented by SEQ ID NO's: 6, 16, 20, 58, 71, 73 or 75 is also provided. The antibody is suitably used to detect expression levels of chimeric DNA according to the invention in host cells and multicellular hosts, preferably plants, capable of producing a protein according to the invention.

The invention also provides a nucleic acid sequence obtainable from a gene encoding a protein according to the invention, said nucleic acid

sequence having tissue-specific transcriptional regulatory activity in a plant. The invention specifically provides a nucleic acid sequence obtainable from the region upstream of the translational initiation site of said gene, preferably at least 500 nucleotides immediately upstream of the translational initiation site of said gene.

DESCRIPTION OF THE FIGURES

Figure 1: SDS-PAGE (12.5%) of the different purification steps of MS59 sunflower protein. Mw= molecular weight markers; 1= crude sunflower protein extract after gel filtration (G25); 2= protein fraction bound to cation exchange chromatography (S-sepharose); 3= pool of active fractions after cation exchange chromatography (Mono S); 4= flow through from hydrophobic interaction chromatography (phenyl superose); 5= active fractions after gel filtration.

Figure 2: SDS-PAGE (12.5%) of different fractions (number 6 to 16) of the gelfiltration (SD75) column. Fraction 10 to 15 was tested in 3 dilutions for growth inhibition on *Phytophthora infestans* (PANEL A) and on *Pythium ultimum* (PANEL B)

Figure 3: SDS-PAGE (12.5%) of fractions eluted from nine gel slices (lane 1 to 9) of a native PAGE in which a MS59 containing SD75 fraction (SD75 fraction 13)was separated. Right panel: SDS-PAGE (12.5%) with SD75 fraction 13 (L) and two fractions of elution experiment fraction 2 (with MS59) and fraction 5 (with a ~30 kD protein). Bottom panel: growth inhibition of *Phytophthora infestans* tested with elution fraction 1 to 6, with 5 µl and 1 µl added per well.

Figure 4: Microscopical analysis of an *in vitro* fungal inhibition assay 24 hours after addition of *Phytophthora infestans* zoosporangia to PDA medium. Left panel: control incubation, only MES buffer was added. Right panel:*E. coli*-produced MS59 in MES buffer was added to the incubation.

Figure 5: Microscopical analysis of an *in vitro* fungal inhibition assay 24 hours after addition of *Pythium ultimum* hyphal fragments to PDA medium. Left panel: control incubation, only MES buffer was added. Right panel: *E. coli*-produced MS59 in MES buffer was added to the incubation.

Figure 6: (A). SD 75 gelfiltration profile of WL64. WL64 eluates at fractions 13, 14, 15. Molecular weight markers are indicated above the arrows at the top of the plot. X-axis: fraction number. Y-axis: A280.
(B). Coomassie stained 12.5% SDS-PAGE gel of fractions 11-17 of the SD 75 gelfiltration profile. Molecular weight markers are indicated on the right and are in kDa. The protein bands that correlate with antifungal activity are indicated between the arrows.
(C). *In vitro* antifungal assay. Ten microlitres of the respective fractions (500 µl total) were used to screen the growth inhibition of *Rhizoctonia solani* hyphal fragments.

Figure 7: Coomassie stained 12.5% SDS-PAGE gel of the purification of WL64. Lane 1, lettuce extract; lane 2, HIC peak; lane 3, Source S peak; lane 4, Mono S peak; lane 5, SD 75 peak; lane 6, Mono P peak. Molecular weight markers are indicated on both sides of the figure and are in kDa.

Figure 8: (A). Lineweaver-Burk plot of MS59 (open diamonds), WL64 (closed circles), and GOX (open squares) oxidase activities with glucose as substrate. Amounts of protein per assay were 17, 29, and 45 ng for MS59, WL64 and GOX respectively.
(B). Lineweaver-Burk plot of MS59 (open diamonds), WL64 (closed circles), and GOX (open squares) oxidase activities with fungal cell walls as substrate. Amounts of protein per assay were 17, 29, and 225 ng for MS59, WL64 and GOX respectively.

Figure 9: Substrate specificity for the oxidase activities of MS59 (dotted bars), WL64 (diagonal striped bars), and GOX (filled bars).

Figure 10: Alignment of the proteins of the invention MS59, WL64 and the two homologues from *A. thaliana* At26 (SEQ ID NO: 71) and At27 (SEQ ID NO: 75) (with the known berberine bridge enzymes (EcBBE and PsBBE). Conserved changes are denoted in gray, while areas of identity (3 of the 6 amino acids identical) are given in black.

DETAILED DESCRIPTION OF THE INVENTION

The antifungal effect of the protein(s) of the invention has been demonstrated in *in vitro* assays for the following fungi; *Phytophthora infestans, Phytophthora cactorum, Phytophtora nicotiana, Phytophthora megasperma, Pythium ultimum, Pythium sylvaticum, Pythium violae, Pythium paroecandrum, Rhizoctonia solani, Tanatephorus cucumeris, Helicobasidium purpureum, Sclerotium cepivorum, Pichia pastoris* and *Botrytis cinerea* for purposes of illustration. It will be clear, that the use of the protein(s) of the invention, or DNA encoding therefore, for use in a process of combating fungi is not limited to the mentioned fungi. There is no reason to assume that the protein(s) according to the invention do not possess antifungal activity against a far broader range of fungi than those tested here, especially in the class of Oomycetes.

Although the invention is illustrated in detail for transgenic tomato, tobacco, carrot, potato and *Brassica napus* plants, it should be understood that any plant species that is subject to some form of fungal attack, especially from the fungi mentioned above, may be provided with one or more plant expressible gene constructs, which when expressed overproduce the protein(s) of the invention in said plant in order to decrease the rate of infectivity and/or the effects of such attack. The invention can even be practiced in plant species that are presently not amenable for transformation, as the amenability of such species is just a matter of time and because transformation as such is of no relevance for the principles underlying the invention. Hence, plants for the purpose of this description shall include angiosperms as well as gymnosperms, monocotyledonous as well as dicotyledonous plants, be they for feed, food or industrial processing purposes; included are plants used for any agricultural or horticultural purpose including forestry and flower culture, as well as home gardening or indoor gardening, or other decorative purposes.

The protein according to the present invention may be obtained by isolating it from any suitable plant source material containing it. A particularly suitable source comprises leaves of the sunflower (*Helianthus*) and leaves of lettuce (*Lactuca sativa* cv. Lollo bionda). The presence of antifungal proteins according to the invention in plant source material can readily be determined for any plant species by making plant extracts from those species and testing those extracts for the presence of antifungal activity using *in vitro* antifungal assays as described herein, further fractionating the obtained samples by any suitable protein fractionation technique in conjunction with the *in vitro* assay until an antifungal fraction is obtained which comprises an approximately 55-65 kDa protein, internally denoted as MS59 or its homologue WL64, which in isolated form shows antifungal activity. Especially, fractions may be tested for antifungal activity on Oomycetes, for example, *Phytophthora* or *Pythium ultimum* and the like, or other fungi, such as the Basidiomycetes, Ascomycetes, Zygomycetes or other classes or subclasses.

Alternatively, antifungal proteins according to the invention may be obtained by cloning DNA comprising an open reading frame capable of encoding said protein, or the precursor thereof, linking said open reading frame to a transcriptional, and optionally a translational initiation and transcriptional termination region, inserting said DNA into a suitable host cell and allowing said host cell to produce said protein. Subsequently, the protein may be recovered from said host cells, preferably after secretion of the protein into the culture medium by said host cells. Alternatively, said host cells may be used directly in a process of combating fungal pathogens according to the invention as a pesticidal acceptable composition.

Host cells suitable for use in a process of obtaining a protein according to the invention may be selected from prokaryotic microbial hosts, such as bacteria *e.g. Agrobacterium*, *Bacillus*, Cyanobacteria, *E.coli* , *Pseudomonas*, and the like, as well as eukaryotic hosts including yeasts, *e.g. Saccharomyces cerevisiae*, fungi, *e.g. Trichoderma* and plant cells, including protoplasts.

In a method of retarding the growth of the fungi on or in the neighbourhood of the plant leaves, host cells may suitably be selected from any species routinely used as biological fungicides.

Also the proteins can be produced by microorganisms, harvested and applied in a agrochemical formulation.

The word protein means a sequence of amino acids connected trough peptide bonds. Polypeptides or peptides are also considered to be proteins. Muteins of the protein of the invention are proteins that are obtained from the proteins depicted in the sequence listing by replacing, adding and/or deleting one or more amino acids, while still retaining their antifungal activity. Such muteins can readily be made by protein engineering *in vivo*, e.g. by changing the open reading frame capable of encoding the antifungal protein such that the amino acid sequence is thereby affected. As long as the changes in the amino acid sequences do not altogether abolish the antifungal activity such muteins are embraced in the present invention.

The present invention provides a chimeric DNA sequence which comprises an open reading frame capable of encoding a protein according to the invention. The expression chimeric DNA sequence shall mean to comprise any DNA sequence which comprises DNA sequences not naturally found in nature. For instance, chimeric DNA shall mean to comprise DNA comprising the said open reading frame in a non-natural location of the plant genome, notwithstanding the fact that said plant genome normally contains a copy of the said open reading frame in its natural chromosomal location. Similarly, the said open reading frame may be incorporated in the plant genome wherein it is not naturally found, or in a replicon or vector where it is not naturally found, such as a bacterial plasmid or a viral vector. Chimeric DNA shall not be limited to DNA molecules which are replicable in a host, but shall also mean to comprise DNA capable of being ligated into a replicon, for instance by virtue of specific adaptor sequences, physically linked to the open reading frame according to the invention. The open reading frame may or may not be linked to its natural upstream and downstream regulatory elements.

The open reading frame may be derived from a genomic library. In this latter it may contain one or more introns separating the exons making up the open reading frame that encodes a protein according to the invention. The open reading frame may also be encoded by one uninterrupted exon, or by a cDNA to the mRNA encoding a protein according to the invention. Open reading frames according to the invention also comprise those in which one or more introns have been

artificially removed or added. Each of these variants is embraced by the present invention.

Also part of the invention are chimeric DNA sequences coding for an antifungal protein which comprise one or more of the EST-sequences shown in SEQ ID NO's: 21 to 48. As can be derived from the sequence listings these EST's for which no function was hitherto known share a considerable homology with the DNA sequence coding for the proteins isolated from *Helianthus* and *Lactuca*.

Another part of the invention is formed by the intrinsic activity of the proteins of the invention. They have been found to be carbohydrate oxidases, capable of oxidating a large number of different mono- and di-saccharides. The substrate specificity resembles the specificity of the enzyme hexose oxidase (EC 1.1.3.5), also known as D-hexose: oxygen 1-oxidoreductase. They have also been shown able to oxidise a purified mixture of fungal (*Rhizoctonia*-derived) cell wall components. It is believed that this oxidative capacity confers the antifungal properties to the proteins. In literature there is one example of an antifungal oxidase, the glucose oxidase from the fungus *Aspergillus* (WO 95/14784). The proteins of this invention, however, show a broader substrate spectrum like hexose oxidase and have a lower Km for the substrate.

From homology searches it has been found that some parts of the amino acid sequence of the proteins of the invention are more conserved and are related to sequences commonly found in oxidases. The highest homology has been found with reticuline oxidase, which enzyme is known from the family of *Papaveraceae* (Facchini, P.J. *et al.*, Plant Physiol. 112, 1669-1677, 1996).

In order to be capable of being expressed in a host cell a chimeric DNA according to the invention will usually be provided with regulatory elements enabling it to be recognised by the biochemical machinery of the host and allowing for the open reading frame to be transcribed and/or translated in the host. It will usually comprise a transcriptional initiation region which may be suitably derived from any gene capable of being expressed in the host cell of choice, as well as a translational initiation region for ribosome recognition and attachment. In eukaryotic cells, an expression cassette usually comprises in addition a transcriptional termination region located downstream of said open reading frame, allowing transcription to

terminate and polyadenylation of the primary transcript to occur. In addition, the codon usage may be adapted to accepted codon usage of the host of choice. The principles governing the expression of a chimeric DNA construct in a chosen host cell are commonly understood by those of ordinary skill in the art and the construction of expressible chimeric DNA constructs is now routine for any sort of host cell, be it prokaryotic or eukaryotic.

In order for the open reading frame to be maintained in a host cell it will usually be provided in the form of a replicon comprising said open reading frame according to the invention linked to DNA which is recognised and replicated by the chosen host cell. Accordingly, the selection of the replicon is determined largely by the host cell of choice. Such principles as govern the selection of suitable replicons for a particular chosen host are well within the realm of the ordinary skilled person in the art.

A special type of replicon is one capable of transferring itself, or a part thereof, to another host cell, such as a plant cell, thereby co-transferring the open reading frame according to the invention to said plant cell. Replicons with such capability are herein referred to as vectors. An example of such vector is a Ti-plasmid vector which, when present in a suitable host, such as *Agrobacterium tumefaciens*, is capable of transferring part of itself, the so-called T-region, to a plant cell. Different types of Ti-plasmid vectors (*vide:* EP 0 116 718 B1) are now routinely being used to transfer chimeric DNA sequences into plant cells, or protoplasts, from which new plants may be generated which stably incorporate said chimeric DNA in their genomes. A particularly preferred form of Ti-plasmid vectors are the so-called binary vectors as claimed in (EP 0 120 516 B1 and US 4,940,838). Other suitable vectors, which may be used to introduce DNA according to the invention into a plant host, may be selected from the viral vectors, *e.g.* non-integrative plant viral vectors, such as derivable from the double stranded plant viruses (*e.g.* CaMV) and single stranded viruses, gemini viruses and the like. The use of such vectors may be advantageous, particularly when it is difficult to stably transform the plant host. Such may be the case with woody species, especially trees and vines.

The expression "host cells incorporating a chimeric DNA sequence according to the invention in their genome" shall mean to comprise

cells, as well as multicellular organisms comprising such cells, or essentially consisting of such cells, which stably incorporate said chimeric DNA into their genome thereby maintaining the chimeric DNA, and preferably transmitting a copy of such chimeric DNA to progeny cells, be it through mitosis or meiosis. According to a preferred embodiment of the invention plants are provided, which essentially consist of cells which incorporate one or more copies of said chimeric DNA into their genome, and which are capable of transmitting a copy or copies to their progeny, preferably in a Mendelian fashion. By virtue of the transcription and translation of the chimeric DNA according to the invention in some or all of the plant's cells, those cells that produce the antifungal protein will show enhanced resistance to fungal infections, especially to *Phytophthora* infections. Although the principles as indicated above govern transcription of DNA in plant cells are not always understood, the creation of chimeric DNA capable of being expressed in substantially a constitutive fashion, that is, in substantially most cell types of the plant and substantially without serious temporal and/or developmental restrictions, is now routine. Transcription initiation regions routinely in use for that purpose are promoters obtainable from the cauliflower mosaic virus, notably the 35S RNA and 19S RNA transcript promoters and the so-called T-DNA promoters of *Agrobacterium tumefaciens*, in particular to be mentioned are the nopaline synthase promoter, octopine synthase promoter (as disclosed in EP 0 122 791 B1) and the mannopine synthase promoter. In addition plant promoters may be used, which may be substantially constitutive, such as the rice actin gene promoter, or *e.g.* organ-specific, such as the root-specific promoter. Alternatively, pathogen-inducible promoters may be used such as the PRP1 promoter (also named gst1 promoter) obtainable from potato (Martini N. *et al.* (1993), Mol. Gen. Genet. 263, 179-186). The choice of the promoter is not essential, although it must be said that constitutive high-level promoters are slightly preferred. It is further known that duplication of certain elements, so-called enhancers, may considerably enhance the expression level of the DNA under its regime (*vide* for instance: Kay R. *et al.* (1987), Science 236, 1299-1302: the duplication of the sequence between -343 and -90 of the CaMV 35S promoter increases the activity of that promoter). In addition to the 35S promoter, singly or doubly enhanced, examples of

high-level promoters are the light-inducible ribulose bisphosphate carboxylase small subunit (*rbc*SSU) promoter and the chlorophyll a/b binding protein (Cab) promoter. Also envisaged by the present invention are hybrid promoters, which comprise elements of different promoter regions physically linked. A well known example thereof is the so-called CaMV enhanced mannopine synthase promoter (US Patent 5,106,739), which comprises elements of the mannopine synthase promoter linked to the CaMV enhancer.

As regards the necessity of a transcriptional terminator region, it is generally believed that such a region enhances the reliability as well as the efficiency of transcription in plant cells. Use thereof is therefore strongly preferred in the context of the present invention.

As regards the applicability of the invention in different plant species, it has to be mentioned that one particular embodiment of the invention is merely illustrated with transgenic tomato and tobacco plants as an example, the actual applicability being in fact not limited to these plant species. Any plant species that is subject to some form of fungal attack, in particular by Oomycetes such as *Phytophthora infestans*, may be treated with proteins according to the invention, or preferably, be provided with a chimeric DNA sequence according to the invention, allowing the protein to be produced in some or all of the plant's cells.

Although some of the embodiments of the invention may not be practicable at present, *e.g.* because some plant species are as yet recalcitrant to genetic transformation, the practicing of the invention in such plant species is merely a matter of time and not a matter of principle, because the amenability to genetic transformation as such is of no relevance to the underlying embodiment of the invention.

Transformation of plant species is now routine for an impressive number of plant species, including both the *Dicotyledoneae* as well as the *Monocotyledoneae*. In principle any transformation method may be used to introduce chimeric DNA according to the invention into a suitable ancestor cell, as long as the cells are capable of being regenerated into whole plants. Methods may suitably be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. *et al.*, 1982, Nature 296, 72-74; Negrutiu I. *et al*, June 1987, Plant Mol.

Biol. 8, 363-373), electroporation of protoplasts (Shillito R.D. *et al.*, 1985 Bio/Technol. 3, 1099-1102), microinjection into plant material (Crossway A. *et al.*, 1986, Mol. Gen. Genet. 202, 179-185), (DNA or RNA-coated) particle bombardment of various plant material (Klein T.M. *et al.*, 1987, Nature 327, 70), infection with (non-integrative) viruses and the like. A preferred method according to the invention comprises *Agrobacterium*-mediated DNA transfer. Especially preferred is the use of the so-called binary vector technology as disclosed in EP A 120 516 and U.S. Patent 4,940,838).

Tomato transformation is preferably done essentially as described by Van Roekel et al. (Van Roekel, J.S.C., Damm, B., Melchers, L.S., Hoekema, A. (1993). Factors influencing transformation frequency of tomato (*Lycopersicon esculentum*). Plant Cell Reports, 12, 644-647). Potato transformation is preferably done essentially as described by Hoekema et al. (Hoekema, A., Huisman, M.J., Molendijk, L., van den Elzen, P.J.M., and Cornelissen, B.J.C. (1989). The genetic engineering of two commercial potato cultivars for resistance to potato virus X. Bio/Technology 7, 273-278).

Generally, after transformation plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant expressible genes co-transferred with the nucleic acid sequence encoding the protein according to the invention, whereafter the transformed material is regenerated into a whole plant.

Although considered somewhat more recalcitrant towards genetic transformation, monocotyledonous plants are amenable to transformation and fertile transgenic plants can be regenerated from transformed cells or embryos, or other plant material. Presently, preferred methods for transformation of monocots are microprojectile bombardment of embryos, explants or suspension cells, and direct DNA uptake or electroporation (Shimamoto, *et al*, 1989, Nature 338, 274-276). Transgenic maize plants have been obtained by introducing the *Streptomyces hygroscopicus bar*-gene, which encodes phosphinothricin acetyltransferase (an enzyme which inactivates the herbicide phosphinothricin), into embryogenic cells of a maize suspension culture by microprojectile bombardment (Gordon-Kamm, 1990, Plant Cell, 2, 603-618). The introduction of genetic material into aleurone protoplasts of other monocot crops such as wheat and barley has been reported (Lee, 1989, Plant Mol. Biol. 13, 21-30). Wheat plants have

been regenerated from embryogenic suspension culture by selecting only the aged compact and nodular embryogenic callus tissues for the establishment of the embryogenic suspension cultures (Vasil, 1990 Bio/Technol. 8, 429-434). The combination with transformation systems for these crops enables the application of the present invention to monocots.

Monocotyledonous plants, including commercially important crops such as rice and corn are also amenable to DNA transfer by *Agrobacterium* strains (*vide* WO 94/00977; EP 0 159 418 B1; Gould J, Michael D, Hasegawa O, Ulian EC, Peterson G, Smith RH, (1991) Plant. Physiol. 95, 426-434).

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the chimeric DNA according to the invention, copy number and/or genomic organization. In addition, or alternatively, expression levels of the newly introduced DNA may be undertaken, using Northern and/or Western analysis, techniques well known to persons having ordinary skill in the art. After the initial analysis, which is optional, transformed plants showing the desired copy number and expression level of the newly introduced chimeric DNA according to the invention may be tested for resistance levels against a pathogen susceptible to the protein according to the invention, such as *Phytophthora infestans*. Alternatively, the selected plants may be subjected to another round of transformation, for instance to introduce further genes, such as genes encoding chitinases, glucanases, osmotins, magainins or the like, in order to enhance resistance levels, or broaden the resistance to other fungi found not to be susceptible to the protein according to the invention in an *in vitro* assay as described herein.

Other evaluations may include the testing of fungal resistance under field conditions, checking fertility, yield, and other characteristics. Such testing is now routinely performed by persons having ordinary skill in the art.

Following such evaluations, the transformed plants may be grown directly, but usually they may be used as parental lines in the breeding of new varieties or in the creation of hybrids and the like.

Many plant proteins exhibit antifungal effects, some however do not do so as such, but yield a significant synergistic antifungal

effect if used in combination with other plant proteins. In European Patent Application 440 304 A1 it was disclosed that simultaneous relative over-expression of a plant expressible glucanase gene in conjunction with a basic chitinase from tobacco in transgenic plants results in a higher level of resistance to fungi than in plants expressing a plant expressible class-I chitinase alone.

Both chitinases, glucanases, osmotins, magainins and the new antifungal protein according to the invention accumulate in infected plant tissues upon an incompatible pathogen-plant interaction. From this observation and the fact that several proteins are found to synergise each others antifungal effects, we envision, that the antifungal protein according to the invention may be suitably used in conjunction with other proteins that are associated with pathogen resistance.

Examples of proteins that may be used in combination with the proteins according to the invention include, but are not limited to, ß 1,3-glucanases and chitinases which are obtainable from barley (Swegle M. *et al.*, 1989, Plant Mol. Biol. 12, 403-412; Balance G.M. *et al.*, 1976, Can. J. Plant Sci. 56, 459-466 ; Hoj P.B. *et al.*, 1988, FEBS Lett. 230, 67-71; Hoj P.B. *et al.*, 1989, Plant Mol. Biol. 13, 31-42 1989), bean (Boller T. *et al.*, 1983, Planta 157, 22-31; Broglie K.E. *et al.* 1986, Proc. Natl. Acad. Sci. USA 83, 6820-6824; Vögeli U. *et al.*, 1988 Planta 174, 364-372); Mauch F. & Staehelin L.A., 1989, Plant Cell 1, 447-457); cucumber (Motraux J.P. & Boller T. (1986), Physiol. Mol. Plant Pathol. 28, 161-169); leek (Spanu P. *et al.*, 1989, Planta 177, 447-455); maize (Nasser W. *et al.*, 1988, Plant Mol. Biol. 11, 529-538), oat (Fink W. *et al.*, 1988, Plant Physiol. 88, 270-275), pea (Mauch F. *et al.* 1984, Plant Physiol. 76, 607-611; Mauch F. *et al.*, 1988, Plant Physiol. 87, 325-333), poplar (Parsons, T.J. *et al*, 1989, Proc. Natl. Acad. Sci. USA 86, 7895-7899), potato (Gaynor J.J. 1988, Nucl. Acids Res. 16, 5210; Kombrink E. *et al.* 1988, Proc. Natl. Acad. Sci. USA 85, 782-786; Laflamme D. and Roxby R., 1989, Plant Mol. Biol. 13, 249-250), tobacco (*e.g.* Legrand M. *et al.* 1987, Proc. Natl. Acad. Sci. USA 84, 6750-6754; Shinshi H. *et al.* 1987, Proc. Natl. Acad. Sci. USA 84, 89-93), tomato (Joosten M.H.A. & De Wit P.J.G.M. 1989, Plant Physiol. 89, 945-951), wheat (Molano J. *et al.*, 1979, J. Biol. Chem. 254, 4901-4907), and the like.

To obtain transgenic plants capable of constitutively expressing more than one chimeric gene, a number of alternatives are available including the following:

A. The use of DNA, *e.g* a T-DNA on a binary plasmid, with a number of modified genes physically coupled to a selectable marker gene. The advantage of this method is that the chimeric genes are physically coupled and therefore migrate as a single Mendelian locus.

B. Cross-pollination of transgenic plants each already capable of expressing one or more chimeric genes, preferably coupled to a selectable marker gene, with pollen from a transgenic plant which contains one or more chimeric genes coupled to another selectable marker. Afterwards the seed, which is obtained by this crossing, maybe selected on the basis of the presence of the two selectable markers, or on the basis of the presence of the chimeric genes themselves. The plants obtained from the selected seeds can afterwards be used for further crossing. In principle the chimeric genes are not on a single locus and the genes may therefore segregate as independent loci.

C. The use of a number of a plurality chimeric DNA molecules, *e.g.* plasmids, each having one or more chimeric genes and a selectable marker. If the frequency of co-transformation is high, then selection on the basis of only one marker is sufficient. In other cases, the selection on the basis of more than one marker is preferred.

D. Consecutive transformation of transgenic plants already containing a first, second, (etc), chimeric gene with new chimeric DNA, optionally comprising a selectable marker gene. As in method B,the chimeric genes are in principle not on a single locus and the chimeric genes may therefore segregate as independent loci.

E. Combinations of the above mentioned strategies.

The actual strategy may depend on several considerations as maybe easily determined such as the purpose of the parental lines (direct growing, use in a breeding programme, use to produce hybrids) but is not critical with respect to the described invention.

In this context it should be emphasised that plants already containing chimeric DNA capable of encoding antifungal proteins may form a suitable genetic background for introducing chimeric DNA according to the invention, for instance in order to enhance resistance levels, or broaden the resistance. The cloning of other genes corresponding to proteins that can suitably be used in

combination with DNA, and the obtention of transgenic plants, capable of relatively over-expressing same, as well as the assessment of their effect on pathogen resistance *in planta*, is now within the scope of the ordinary skilled person in the art.

The obtention of transgenic plants capable of expressing, or relatively over-expressing, proteins according to the invention is a preferred method for counteracting the damages caused by fungi, such as Oomycetes like *Phytophthora infestans*, as will be clear from the above description. However, the invention is not limited thereto. The invention clearly envisions also the use of the proteins according to the invention as such, preferably in the form of a fungicidal composition. Fungicidal composition include those in which the protein is formulated as such, but also in the form of host cells, such as bacterial cells, capable of producing the protein thereby causing the pathogen to be contacted with the protein. Suitable host cells may for instance be selected from harmless bacteria and fungi, preferably those that are capable of colonising roots and/or leaves of plants. Example of bacterial hosts that may be used in a method according to the invention are strains of *Agrobacterium*, *Arthrobacter*, *Azospyrillum*, *Pseudomonas*, *Rhizobacterium*, and the like, optionally after having been made suitable for that purpose.

Compositions containing antifungal proteins according to the invention may comprise in addition thereto, osmotin-like proteins as defined in WO91/18984. Independently, the invention provides antifungal compositions which further comprise inhibitory agents such as classical fungal antibiotics, SAFPs and chemical fungicides such as polyoxines, nikkomycines, carboxymides, aromatic carbohydrates, carboxines, morpholines, inhibitors of sterol biosynthesis, organophosphorus compounds, enzymes such as glucanases, chitinases, lysozymes and the like. Either per se, or in combination with other active constituents, the antifungal protein of the invention should be applied in concentrations between 1 ng/ml and 1 mg/ml, preferably between 2 ng/ml and 0.1 mg/ml, within pH boundaries of 3.0 and 9.0. In general it is desired to use buffered preparations, *e.g.* phosphate buffers between 1mM and 1M, preferably between 10 mM and 100mM, in particular between 15 and 50 mM, whereby in case of low buffer concentrations it is desired to add a salt to increase ionic strength, preferably NaCl in concentrations between 1 mM and 1M, preferably 10

mM and 100 mM.

Plants, or parts thereof, which relatively over-express a protein according to the invention, including plant varieties, with improved resistance against fungal diseases, especially diseases caused by Oomycetes like *Phytophthora* and *Pythium* may be grown in the field, in the greenhouse, or at home or elsewhere. Plants or edible parts thereof may be used for animal feed or human consumption, or may be processed for food, feed or other purposes in any form of agriculture or industry. Agriculture shall mean to include horticulture, arboriculture, flower culture, and the like. Industries which may benefit from plant material according to the invention include but are not limited to the pharmaceutical industry, the paper and pulp manufacturing industry, sugar manufacturing industry, feed and food industry, enzyme manufacturers and the like.

The advantages of the plants, or parts thereof, according to the invention are the decreased need for fungicide treatment, thus lowering costs of material, labour, and environmental pollution, or prolonging shelf-life of products (e.g. fruit, seed, and the like) of such plants. Plants for the purpose of this invention shall mean multicellular organisms capable of photosynthesis, and subject to some form of fungal disease. They shall at least include angiosperms as well as gymnosperms, monocotyledonous as well as dicotyledonous plants.

The phrase "plants which relatively over-express a protein" shall mean plants which contain cells expressing a transgene-encoded protein which is either not naturally present in said plant, or if it is present by virtue of an endogenous gene encoding an identical protein, not in the same quantity, or not in the same cells, compartments of cells, tissues or organs of the plant. It is known for instance that proteins which normally accumulate intracellularly may be targeted to the apoplastic space.

According to another aspect of the invention the regulatory region of a plant gene coding for the antifungal protein of the invention may be used to express other heterologous sequences under the control thereof. The use of a regulatory element of at least 1000 bp directly upstream of the gene coding region is sufficient for obtaining expression of any heterologous sequence.

Heterologous sequences in this respect means gene regions not naturally associated to said regulatory region, and they comprise both different gene coding regions, as well as antisense gene regions. Heterologous coding sequences that may be advantageously expressed in the vascular tissue comprise those coding for antipathogenic proteins, *e.g.* insecticidal, bactericidal, fungicidal, and nematicidal proteins. In such a strategy it may prove exceptionally advantageous to select a protein with activity against a pathogen or pest which has a preference for phloem as source of nutrients (*e.g.* aphids), or as entrance to invade the plant. Examples are extensin, lectin, or lipoxidase against aphids (See WO93/04177). Assuming that the regulatory region according to the invention is active in xylem, antifungal proteins may be expressed under the control of said regulatory region to combat *Fusarium, Verticillium, Alternaria* and *Ceratocystus* species.

The use of the regulatory region according to the invention may also be used advantageously to regulate or control phloem transport processes. Numerous other applications will readily occur to those of skill in the art.

The expression of part of (part of) an endogenous gene in the antisense orientation (such as disclosed in EP 0 233 399 A), can effectively down-regulate expression of said endogenous gene, with interesting applications. Moreover, the gene encoding the antifungal protein according to the invention itself may be down-regulated using the antisense approach which may help establishing the nature and function of the protein. The regions responsible for tissue-specific expression may be unravelled further using the GUS-marker in a way analogous to the way illustrated herein.

The following state of the art may be taken into consideration, especially as illustrating the general level of skill in the art to which this invention pertains.
EP-A 392 225 A2; EP-A 440 304 A1; EP-A 460 753 A2; WO90/07001 A1; US Patent 4,940,840.

Yet another part of the invention is directed at the production of a novel oxidative enzyme, capable of oxidising carbohydrates even at low concentrations due to its low Km. Most specifically hexoses are the substrate of the enzymatic activity although also other sugars are

affected to some lesser extent. The enzymes can be isolated from the sources in which they naturally occur (according to the method described in this invention) or they can be isolated from plants or other organisms transformed with an expressible gene encoding the protein. These oxidases can be used in industrial processes for the oxidation of carbohydrates, such as glucose, mannose, galactose, cellobiose, maltose and lactose.

Evaluation of transgenic plants

Subsequently transformed plants are evaluated for the presence of the desired properties and/or the extent to which the desired properties are expressed. A first evaluation may include the level of expression of the newly introduced genes, the level of fungal resistance of the transformed plants, stable heritability of the desired properties, field trials and the like.

Secondly, if desirable, the transformed plants can be crossbred with other varieties, for instance varieties of higher commercial value or varieties in which other desired characteristics have already been introduced, or used for the creation of hybrid seeds, or be subject to another round of transformation and the like.

Synergy

The combination of one of the antifungal protein according to the instant invention and other antifungal proteins of plant or microbial source are predicted to show a drastic synergistic antifungal effect. Similar synergistic antifungal effects were shown if combinations of antifungal CBPs or Chi-V are combined with either ß-1,3-glucanases or chitinases from other plant origins. Apparently, the synergizing effect of combinations of pathogen induced proteins is a more general phenomenon that has important consequences for the engineering of fungal resistant plants.

Plants, or parts thereof of commercial interest, with improved resistance against phytopathogenic fungi can be grown in the field or in greenhouses, and subsequently be used for animal feed, direct consumption by humans, for prolonged storage, used in food- or other industrial processing, and the like. The advantages of the plants, or parts thereof, according to the invention are the decreased need for

fungicide treatment, thus lowering costs of material, labour, and environmental pollution, or prolonged shelf-life of products (e.g. fruit, seed, and the like) of such plants.

EXPERIMENTAL PART

Standard methods for the isolation, manipulation and amplification of DNA, as well as suitable vectors for replication of recombinant DNA, suitable bacterium strains, selection markers, media and the like are described for instance in Maniatis *et al.*, molecular cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press; DNA Cloning: Volumes I and II (D.N. Glover ed. 1985); and in: From Genes To Clones (E.-L. Winnacker ed. 1987).

In vitro antifungal assay

All fungi were cultured on potato dextrose agar (Difco) at 25^{0}C, except *Botrytis cinerea* and *Phoma lingam* which were grown on oat meal agar (Difco) at 25^{0}C. *Phytophthora infestans* was grown on rye agar at 18^{0}C in the dark (Caten and Jinks, 1968). *Botrytis cinerea* and *Phoma lingam* were cultivated under UV. Spores of sporulating fungi were harvested by flooding the agar plates with water. The spore concentration was adjusted to 10,000 sp/mL. In the case of *Rhizoctonia solani* and *Pythium ultimum* liquid shake cultures were grown in potato dextrose broth at 25^{0}C. To prepare inoculum from these shake cultures, mycelium was harvested and vortexed for 1 minute. After passage through a fine sieve, inoculum density was adjusted to 2500 - 5000 fragments, of 1 to 3 cells each, per mL.

In case of sporulating fungi, all were tested both with and without pregerminating the spores before application of the protein samples. In case of non-sporulating fungi, hyphal fragments were used.

The antifungal activity was monitored during purification in a microtiter plate assay using the fungi *Phytophthora infestans* and *Pythium ultimum* according to Woloshuk *et al.*, 1991 or using other fungi in a similar way. In each well of a 24-well microtiter dish 250 µl potato dextrose agar (PDA) was pipetted. Fungal spores in the case of e.g. *Phytophthora infestans* and hyphal fragments in the case of e.g. *Pythium ultimum* were suspended in water and 400-600 spores or 200 fragments in 50 µl were added to the wells. Subsequently 100 µl filter sterilized (0.22 µm filter) protein solution (in 50 mM MES, pH 6.0)

was added. Microtiter dishes were wrapped with Parafilm and incubated at room temperature. At several timepoints after the initiation of incubation the fungus was monitored microscopically for effects of the added protein. After 2-3 days the mycelium of the growing fungus in the wells was stained with lactophenol cotton blue and the extent of growth was estimated.

GI: growth inhibition; a scale of 0 - 4 is used, 0 = no visible inhibition, 1 = weak inhibition (0 to 30%) inhibition, 2 = moderate (30 to 60%) inhibition, 3 = strong (60 to 90%) inhibition, 4 = very strong (100%) inhibition.

EXAMPLE 1

Purification of an antifungal protein MS59 from sunflower induced with salicylic acid

Leaves of 7 to 8 weeks old sunflower (*Helianthus annuus* cv. zebulon) plants were sprayed daily for 5 times with 10 mM sodium salicylate. After 3 hours the plants were extensively rinsed with water to remove the sodium salicylate. Three days after the final spray, leaves (400 gram) were harvested into liquid nitrogen and homogenized at 4°C in 500 ml 0.5 M NaOAc pH5.2, and 4 gram active carbon, using a Waring blender. The homogenate was filtered over four layers of cheese cloth and subsequently the filtrate was centrifuged for 50 minutes at 20,000 g at 4°C and desalted by passage through a Sephadex G25 column (medium course; Pharmacia), length 60 cm, diameter 11.5 cm, equilibrated in 40 mM NaOAc pH5.2. The desalted protein solution was stored overnight at 4°C and subsequently centrifuged for 45 minutes at 20,000 g at 4°C. The supernatant was passed through a S-sephadex (Fast-flow, Pharmacia) column, length 5 cm, diameter 5 cm, which was equilibrated with 40 mM NaOAc pH 5.2. The column was washed with the above mentioned buffer (flow rate 400 to 500 ml/hr) until the OD_{280} dropped to zero. The bound proteins were eluted using 400 mM NaCl in 200 ml of the above mentioned buffer.

After dialysis against 50 mM MES pH 6.0 the eluate was analyzed for antifungal activity. Antifungal activity was monitored in a microtiter plate assay using the fungus *Phytophthora infestans* and *Pythium ultimum*. See above for details concerning *in vitro* assaying. Subsequently, cationexchange chromatography was reapplied whereby the eluate was passed through an FPLC Mono-S HR 5/5 (Pharmacia) and eluted

with a linear gradient from 0 to 400 mM NaCl. All fractions were analyzed by electrophoresis (Laemmli (1970), Nature 227:680-685) using a 12.5% polyacrylamide gel in the presence of sodium dodecyl sulphate (SDS), using prestained molecular weight markers (15-105 kDa) as reference. Additionally, of all fractions antifungal activity towards *Phytophthora infestans* and *Pythium ultimum* was monitored. Antifungal activity eluted from the column between 45-60 mM NaCl and in all active fractions a 59 kD band was visible. Fractions containing the antifungal activity were pooled and dialysed to 1 M ammonium sulphate in 50 mM potassium phosphate, pH 7. The pool was subjected to hydrophobic interaction chromatography, whereby the sample was applied to an FPLC Phenyl Superose HR 5/5 (Pharmacia) equilibrated in the same buffer and eluted with a linear decreasing gradient from 1 to 0 M ammonium sulphate in 50 mM potassium phosphate, pH 7. As above again all fractions were analyzed on SDS-PAGE and monitored for antifungal activity. Also the pool of proteins not capable of binding to this column (Flow Through, FT) was thus analyzed at the conditions chosen here. Antifungal activity was present most abundantly in the FT and secondly also in the fractions eluting between 0.76 and 0.45 mM ammonium sulphate. In both cases a 59 kD protein was visible on SDS-PAGE. FT and the gradient fractions were separately dialysed to 50 mM MES, 0.2 M NaCl and separately chromatographed on a FPLC Superdex 75 HR 10/30 column (Pharmacia) equilibrated to the same buffer. Proteins elute from this column according to their molecular size. In both cases again the presence of a 59 kD protein coincided with antifungal activity towards *Phytophthora infestans* and *Pythium ultimum* as judged from SDS-PAGE and *in vitro* antifungal assays. The 59 kD protein present in the FT of the hydrophobic interaction column was most abundant and termed MS59 and its purification is visualized in Figure 1. Results of its separation over the gelfiltration column and subsequent analysis both on SDS-PAGE and on *Phytophthora infestans* is shown in Figure 2. Several characteristics (antifungal activity, chromatographical properties, molecular mass) of the gradient protein and MS59 indicate that the two proteins are very similar.

To characterize MS59 further its amino acid sequence was partially determined. Therefore, MS59 was separated in the presence of 0.1 mM thioglycolate in the upper reservoir buffer and SDS on a 12.5% polyacrylamide gel, which was prerun for 2 hours at 50 V with 0.05 mM

glutathione in the upper reservoir buffer. The gel was stained with 5% (w/v) Serva Blue G in 45% (v/v) methanol and 10% acetic acid for 30 minutes and destained in 20% (v/v) acetic acid for 30 minutes and the 59 kDa band was cut out and sequenced using Edman degradation on an Applied Biosystems 477A protein sequencer according to the protocol provided by the manufacturer. N-terminal amino acid sequencing of MS59 revealed that the N-terminus was blocked. To obtain internal sequences, MS59 was digested with trypsin. Trypsin cleaves protein at arginine and lysine residues. The digestion products were separated on a reversed-phase column and analyzed by Edman degradation. Two tryptic fragments were sequenced: Pep1 and Pep2. Of Pep1 25 amino acid residues were identified: S-I-N-V-D-I-E-Q-E-T-A-W-V-Q-A-G-A-T-L-G-E-V-Y-Y-R (SEQ ID NO: 1).

The amino acid sequence is given using the one-letter code. Of Pep2 a further 25 amino acid residues were identified: D-P-S-F-P-I-T-G-E-V-Y-T-P-G-(?)-S-S-F-P-T-V-L-Q-N-Y (SEQ ID NO: 2).

The amino acid residue between brackets could not be identified unambiguously.

EXAMPLE 2

Elution of antifungal protein from native PAGE and subsequent testing

It is obvious from Figures 1 and 2 that MS59 is not completely pure. To further ensure that indeed the 59 kDa protein is responsible for the observed antifungal activity, the fraction containing the peak amount of 59 kDa was electrophoresed on a native gel, using the same system as described above however without SDS and without boiling the samples before loading. The gel lane was sliced in 0.5 cm horizontal pieces and each piece was eluted individually for 48 hours in 50 mM Mes, pH 6. After centrifugation the resulting supernatant was analyzed both on SDS-PAGE and *in vitro* for antifungal activity. Results are shown in Figure 3. Only in those fractions containing MS59, was antifungal activity observed against *Phytophthora infestans* and *Pythium ultimum*.

EXAMPLE 3

In vitro antifungal assays on non-Oocymetes

In vitro fungal assays were performed as described in the general experimental part. As positive control *Phytophthora infestans* was tested. The peak of MS59 is located in fraction 4. Results are shown in Table 1.

Table 1. Antifungal effects of MS59 containing fractions from Mono-S, pH 6

fungus	spore stage *)	fraction number 1	2	3	4	5	6	7/8
Fusarium oxysporum	spore	0	0	0	0	0	0	0	0
	germl.	2	2	2	2	3	3.5	3.5	3.5
Fusarium solani	spore	0	0	0	0	0	0	0	0
Phytophthora infestans	spore	0	2	2	4	3.5	2	1	0
Phytophthora nicotianae	hyph	0	1	2	4	4	2	1	0
Phytophthora cactorum	hyph	0	0	2	4	4	1	1	0
Pythium ultimum	hyph	0	0	0	4	4	0	0	0
Pythium sylvaticum	hyph	0	0	0	2	1	0	0	0
Pythium paroecandrum	hyph	0	0	0	2	2	0	0	0

*) spore = no pregermination, germl = germination until the germtube is 3-5 times the length of the spore, hyph. = hyphal fragments were used as starting inoculum.

GI: growth inhibition; a scale of 0 - 4 is used, 0 = no visible growth inhibition, 1 = weak (0 to 30%) inhibition, 2 = moderate (30 to 60%) inhibition, 3 = strong (60 to 90%) inhibition, 4 = very strong (100%) inhibition.

As can be seen *Phytophthora* and *Pythium* spp., appeared very sensitive to MS59.

EXAMPLE 4

Purification of an antifungal protein WL64 from lettuce induced with salicylic acid

Leaves of 7 to 8 weeks old lettuce (*Lactuca sativa* cv. Lollo bionda) plants were sprayed daily with 10 mM salicylate for 4 days. After two hours the plants were extensively rinsed with water to remove the sodium salicylate. On day 5, the leaves were harvested into liquid nitrogen and stored at -80^{0}C until further use.

Lettuce leaves were thawed and homogenized at 4^{0}C in 0.5M NaOAc pH 5.2, 0.1% ß-mercaptoethanol (lettuce : buffer = 1: 1.5 (w/v)), and 10 grams active carbon per kg leaves, using a Waring blender. The homogenate was centrifuged for 60 minutes at 9,000 g at 4^{0}C. The supernatant was subsequently filtered over 10 layers of cheese cloth. The filtrate was brought to 40% saturation with ammonium sulphate and centrifuged for 30 minutes at 9,000 g. The resulting supernatant, containing 85% of protein and >95% of antifungal activity relative to the crude homogenate, was subjected to hydrophobic interaction chromatography.

The supernatant was filtered over a paper filter and applied to a phenyl-sepharose 6FF High sub column (Pharmacia, 100 ml bed volume in a Pharmacia XK 50/20 column) pre-equilibrated with 40% (1.45M) ammonium sulphate in 50mM potassium-phosphate buffer, pH 6.0 (referred to as buffer A) at a flow rate of 10 ml/min or less. The column was washed with at least 10 column volumes of buffer A after which bound protein was eluted with a decreasing salt gradient from 100% buffer A to 20% buffer A (50mM KPi pH 6.0 as buffer B) over a period of 40 min at a flow rate of 10 ml/min, followed by a linear decreasing gradient from 20% A to 0% A (=100% B) over a period of 30 min at the same flow rate. The column was washed for another 45 min with buffer B, after which the elution was completed. One-minute fractions were collected (10 ml/fraction). Fractions 40-75 (called the HIC-peak) contained antifungal activity.

The pooled fractions were concentrated (using a stirred flow cell and a YM 30 kDa membrane (Amicon)) and subsequently 15 times diluted with 25mM sodium acetate, pH 4.5. This solution was applied to a pre-packed Source S column (16/20, Pharmacia) with a flow rate of 10 ml/min. After washing of the column with 5 column volumes of said buffer, protein was eluted from the column with an increasing NaCl

gradient (0-0.4M NaCl in 25mM NaOAc, pH 4.5) over a 60 min period, 2.5 ml/min, 1 min fractions. Fractions were collected in 250µl 1M potassium phosphate, pH 7.0, in order to neutralize the relatively acidic NaOAc buffer. The fractions containing antifungal activity (fractions 25-45 (0.2-0.3M NaCl)) were pooled and are referred to as the Source S-peak.

The Source S-peak was concentrated and buffer exchanged to 25mM NaOAc, pH 4.5, resulting in a fraction of about 10 ml, and subjected to cation-exchange chromatography using a Mono S column (5/5, Pharmacia). The column is eluted with the following NaCl gradients (NaCl in 25mM NaOAc, pH 4.5): 0-5 min, 0-0.1M NaCl; 5-20 min, 0.1-0.16M NaCl; 20-21 min, 0.16-0.25M NaCl; 21-31 min, 0.25M NaCl; 31-32 min, 0.25-1.0M NaCl, followed by 1.0M NaCl for 10 min after which the elution is completed. The antifungal activity eluted from the column during the 0.25M NaCl step (usually fractions 22-30; the Mono-S peak). Flow-rate 1 ml/min, 1ml fractions, collected in 100µl 1M potassium phosphate, pH 7.0.

The Mono S-peak was concentrated to about 0.5-1.0 ml and subjected to gelfiltration chromatography (Superdex 75, 10/30, Pharmacia), with 200mM NaCl in 50mM potassium phosphate, pH 7.0 as the running buffer. The sample volume was 200 µl; flow rate 0.5 ml/min; 0.5 ml/fraction. The antifungal activity elutes from the column at the position of the 66 kDa marker. Comparison of the active fractions (SD 75 peak) with the protein pattern on SDS-PAGE reveals a 64 kDa protein as the most likely candidate for the lettuce-antifungal protein (Figs. 6A-C). This protein was named WL64.

The SD 75-peak was buffer-exchanged to pH 9.5 for chromatophocusing on a Mono P column (Pharmacia) according to the manufacturers instructions. All activity was found in the flow-through of the column (even in the case when the column was equilibrated to pH 11.0) although there was some separation (3 overlapping peaks in flow-through). The flow-rate was 0.5 ml/min; 0.5 ml/fraction. The fractions containing the anti-fungal activity were pooled and buffer-exchanged to 50mM MES, pH 6.0. Coomassie staining of the highest purified protein fraction after SDS-PAGE revealed about 6 protein bands of which two bands of 64 kDa and 55 kDa, were the most prominent ones (Fig. 7). The estimated relative amounts of both proteins in the final fraction was 1/6-1/8 for the 64 kDa protein and 1/2-1/3 for the 55 kDa

protein. Although on gel it is shown that this column clearly contributes to the purification of the 64 kDa protein, the specific activity, as well as the recovery of the protein in the pooled fractions dropped considerably (see table 2).

A representative purification procedure is summarized in table 2.

Table 2. Purification of WL 64

Sample or Column	Protein (mg)	Activity (GI-units)	Spec. act. (GI-u/mg)	Purification (x-fold)	Recovery (%)
Lettuce (1.54 kg)					
Extract	685				
$(NH_4)_2SO_4$ sup	584	101250	173	1	100
HIC	174	44000	253	1.46	43
Source S	38.7	32400	837	4.84	32
Mono S	2.3	8960	3896	22.5	8.8
SD-75	0.452	8200	18142	105	8.1
Mono P	0.137	1752	12788	74	1.7

The activity is represented as growth inhibition units (GI-units). Four GI units represent the amount of protein that results in a growth inhibition of 100% in the *in vitro* assay as described in the general part of the Examples.

EXAMPLE 5

Elution of WL64 from native PAGE and subsequent testing

Since WL64 was not completely pure, it was further investigated wether or not the 64 kDa protein was indeed responsible for the observed antifungal activity. The Mono P fraction containing the peak amount of antifungal activity was submitted to electrophoresis on a native 10% polyacrylamide gel under acidic conditions, in the absence of SDS and ß-mercaptoethanol and without boiling. Two adjacent gel lanes were sliced in 0.3 cm horizontal pieces. One part was used

directly in the antifungal assay, the other part was subjected to SDS-PAGE under denaturing conditions. Growth inhibition clearly correlated to the 64 kDa protein and not to the 55 kDa protein.

EXAMPLE 6

Glycosylation of WL64

WL64, as well as the 55kDa protein are glycosylated as illustrated by binding to concanavalin A and by the DIG-Glycan-detection kit (Boehringer). Both proteins were not sensitive to glycopeptidase-F treatment, indicating that the glycosylation is probably O-linked.

EXAMPLE 7

Amino acid sequencing of WL64

For N-terminal amino acid sequencing an amount of 21 µg of purified protein (representing about 4µg WL64) was separated on a 7.5% polyacrylamide gel and was subsequently blotted onto PVDF membrane. The membrane was stained with 0.1% Serva Blue G in 45% methanol, 10% acetic acid for 5 minutes at room temperature and destained with 45% methanol, 10% acetic acid. The 64 kDa band was cut out and sequenced using Edman degradation on an Applied Biosystems 477A protein sequencer according to the protocol provided by the manufacturer.

For internal protein sequencing 105 µg of purified protein (representing about 20µg WL64) was separated on a 7.5% SDS-polyacrylamide gel. The gel was stained with 0.2% Serva Blue G in 20% methanol, 0.5% acetic acid for 20 min at room temperature and destained with 30% methanol at room temperature for about 1 hour. The 64 kDa band was cut out and the protein was subsequently digested with trypsin. The digestion products were separated on a reverse phase column and analyzed by Edman degradation.

Besides the N-terminal sequence (SEQ ID NO: 49), two tryptic fragments were sequenced (SEQ ID NO: 50 and SEQ ID NO: 51).

SEQ ID NO: 49: Thr-Ser-Thr-Ser-Ile-Ile-Asp-Arg-Phe-Thr-Gln-(Cys/Ser)-Leu-Asn-Asn-Arg-Ala-Asp-Pro-(Ser)-(Phe)-

SEQ ID NO: 50: (Ser)-Ile-(???)-Val-(Ser)-Ile-Glu-Asp-Glu-Thr-Ala-(Trp)-Val-Gln-Ala-Gly-Ala-Thr-Leu-Gly-Glu-Val-Tyr-(Tyr)-

SEQ ID NO: 51: Ala-Asp-Pro-Ser-Phe-Pro-Leu-Ser-Gly-Gln-Leu-Tyr-Thr-Pro-

The amino acid residues between brackets could not be identified unambiguously.

EXAMPLE 8

Anti-fungal activity of MS59 and WL64

Based on the sequence homology between MS59 and WL64, both proteins appear to be very related to each other. This might also be the case for their anti-fungal activity, as well as for their specific activities towards the respective fungi. This hypothesis was tested and the results are summarized in table 3.

Table 3. Anti-fungal activity of MS59 and WL64 [1]

Pathogen	Amount of WL64 needed for complete inhibition (GI 4) (ngram per assay)	Amount of MS59 needed for complete inhibition (GI 4) (ngram per assay)
Phytophthora infestans	10	5
Pythium ultimum	10	5
Rhizoctonia solani	20	10
Tanatephorus cucumeris	20	n.t.
Helicobasidium purpureum	15	7.5
Sclerotium cepivorum	40	20
Pichia pastoris	n.t.	5
Botrytis cinerea	200	10

n.t. = not tested

[1] Antifungal assays were carried out as described in the general experimental part.
Note that the amounts of protein were estimated by means of Coomassie staining on SDS-PAGE gels, meaning that the amounts of protein depicted here are indicative, rather than absolute.

EXAMPLE 9

Oxidase activities

A 50ml culture of *Rhizoctonia solani* in potato dextrose broth was extensively sonicated on ice and subsequently centrifuged at 3,000 g for 20 minutes at 4°C. The resulting supernatant was then centrifuged at 25,000 g for 1 hour. The pellet was washed twice with demineralized water and resuspended in 1 ml water containing 1.0% Triton X-100. In this way a fungal cell wall suspension was obtained.

Oxidase activity was measured utilizing the reagent 4-amino-antipyrine (4-AAP), based on Gallo, 1981 (Gallo, Methods in Enzymology, 71:665-668, 1981). A reaction volume of 500µl contained 50mM potassium phosphate buffer pH 7.0, 25µM FAD, 10mM NaN_3, 0.01% Triton X-100, 6mM 2,4,6,tribromohydroxybenzoic acid, 2mM 4-AAP, and 10 units horseradish peroxidase. Hydrogenperoxide production was measured at 510nm. Known amounts of hydrogen peroxide were included for calibration.

WL64, as well as MS59, performed oxidase activities using the fungal cell wall suspension as substrate. Different substances were subsequently tested as possible substrates, a.o. some carbohydrates and amino acids (see example 10). Glucose, and other carbohydrates were found to serve as substrate for the oxidase activity of both MS59 and WL64.

Since MS59 and WL64 displayed carbohydrate and especially glucose oxidase activity it was investigated whether the fungal cell wall suspension could serve as a substrate for glucose oxidase (GOX) from Aspergillus niger (Sigma, G 2133). This was indeed the case. Kinetic studies showed that MS59, WL64 and GOX display Michaelis-Menten kinetics when glucose is used as substrate, as illustrated by means of a Lineweaver-Burk plot (Fig 8A). The K_m values for MS59 and WL64 were more than one order of magnitude lower than that for GOX: 19.5µM and 23.3µM for WL64 and MS59 respectively and 359µM for GOX. This means that the affinity for glucose is much higher for MS59 and WL64 than that of GOX. The V_{max} values were, however, comparable being 5.7, 16.8, and 9.7µmol H_2O_2/min/mg protein for WL64, MS59 and GOX, respectively.

Kinetic studies using the fungal cell wall suspension as substrate showed Michaelis-Menten kinetics for both MS59 and WL64, but

not for GOX as shown in Fig. 8B. The K_m values for MS59 and WL64 were 4.7µl and 24.3µl respectively, using the suspension described above. The V_{max} values were 22.0 and 11.2µmol H_2O_2/min/mg protein for respectively MS59 and WL64. Since GOX with fungal cell walls as substrate does not show a linear relationship in a Lineweaver-Burk plot, the K_m and V_{max} could not be extrapolated from the plot. The kinetic data are summarized in table 4.

Table 4. Kinetic parameters of MS59, WL64 and glucose oxidase

Enzyme	Glucose		Fungal Cell Wall Suspension	
	K_m (µM)	V_{max} (µmolH_2O_2/min/mg)	K_m (µl)	V_{max} (µmolH_2O_2/min/mg)
MS59	23.3	16.8	4.7	22.0
WL64	19.5	5.7	24.3	11.2
GOX	359	9.7		

EXAMPLE 10

Substrate specificities

Different substances were tested as possible substrates. Among sucrose, sorbitol, fructose, c.m. cellulose, ß-alanine, aspartic acid, chitine, cellulose, glutamate, glycine-glycine, laminarin, and glucose, only the latter served as substrate for at least WL64. Concentrations of the various substrates varied between 5mM and 50mM. It was further investigated whether glucose was the only substrate for MS59 and WL64 or that other carbohydrates could also be oxidized. The enzyme assays were performed as described in Example 9, the substrate concentrations being 50mM. GOX was shown to oxidase glucose exclusively (Fig. 9). Same figure shows that MS59 and WL64 display a much broader substrate specificity, ranging from C_4-sugars to di- and polysaccharides. The highest (and almost equal activities) were obtained with D-glucose, D-mannose, D-galactose, cellobiose, maltose, and lactose (Fig. 9). This range of substrates resembles the range found to be converted by hexose oxidase (EC. 1.1.3.5).

EXAMPLE 11

Identification and characterization of genes homologous to the deduced MS59 nucleotide sequence

Based on the amino acid sequences of pep1 (a.a. 12 to 22 of SEQ ID NO: 1) and pep2 (a.a. 2 to 12 of SEQ ID NO: 2), primers were designated for PCR. Genomic DNA was isolated from sunflower cv. Zebulon and PCR primers 4 (5'AAC TTC TCC IAG IGT IGC ICC IGC TTG IAC CCA3', SEQ ID NO: 3) and 5 (5'GAT CCI TCT TTC CCI ATT ACT GGI GAG GTT TA3', SEQ ID NO: 4) were used to amplify a 354 bp DNA fragment from the sunflower genome with PCR. PCR products corresponding to this fragment size were cloned (SEQ ID NO: 5). Sequence analysis of the product revealed the presence of an uninterrupted Open Reading Frame (ORF) (SEQ ID NO: 6) of which the first and last stretch of amino acids corresponded with the amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2. Several clones sequenced contained point mutations, varying from 1 to 4 in this PCR fragment. All but one of these mutations were silent mutations (nucleotide nr 57 T to C, nucleotide nr 63 C to A, nucleotide nr 225 A to G) which therefore did not alter amino acid sequences encoded. One clone however did contain a point mutation (nucleotide nr 203 G to A) which altered the amino acid sequence at amino acid 68 from Arg to Lys.

A southern blot of sunflower genomic DNA, probed with SEQ ID NO: 5 indicated the existence of multiple homologous sequences in the genome. Using SphI, 6 bands were detected, EcoRV 5 bands, SpeI 3 bands and NdeI 4 bands. With other enzymes 3-4 bands were previously discerned. This analysis suggests the existence of 3 genes with (partial) homology to the ms59 sequences.

New PCR primers were developed based on the non-variable areas between the original PCR primer sequences. Primers: for 3' RACE: 5' CAG GCA GCT GTG GTT TGT GGC 3' (SEQ ID NO: 7),
for 5' RACE: 5' GTC CAC AAT GAA GAA GGG TTG 3' (SEQ ID NO: 8) and for nested 3'RACE: 5' ACG TAG ATA TCG AAC AAG AAA CCG C 3' (SEQ ID NO: 9).

Poly(A) containing RNA was isolated from sunflower leaf material that was induced by spraying 5 times with a 10 mM sodium salicylate solution. cDNA was prepared and 5' and 3' RACE PCR reactions were

performed as described in the instructions of the Marathon™ kit (Clontech laboratories, Inc., Palo Alto, CA). Partial cDNA clones were isolated by 5' and 3' RACE PCR reactions. Sequence analysis confirmed the identity of the partial cDNA clones.

Again new PCR and nested PCR primers were developed based on newly obtained sequence information from cloned 5' and 3' RACE PCR products. Primers for 5'RACE: 5' CTG GGG AAG CCC GTG TAG TAA AGC 3' (SEQ ID NO:11), 5' CGG GAA GTT GCA GAA GAT TGG GTT G 3' (SEQ ID NO:13), for nested 5'RACE: 5' GAG CAA GAG AAG AAG GAG AC 3' (SEQ ID NO:14), for 3' RACE: 5' GCT TTA CTA CAC GGG CTT CCC CAG 3' (SEQ ID NO: 10), and for nested 3' RACE: 5' GGT ACT CCA ACC ACG GCG CTC 3' (SEQ ID NO:12). Four partial cDNA clones were isolated which together encode all of the Open Reading Frame including a putative signal peptide followed by an approximately 59 kDa protein, and 5' and 3' UTR's (untranslated regions)(SEQ ID NO: 15). A full length cDNA clone of 1784 bp, of which the ORF (pos. 21 to pos. 1608) encodes 529 amino acid residues (SEQ ID NO:16), could be assembled out of these four partial cDNA clones and the PCR fragment mentioned above (SEQ ID NO: 5).
The amino-terminal signal sequence (Von Heijne *et al.*, 1983 and Von Heijne, 1985) is not likely fully presented within the first 19 amino acid residues. A prediction of the putative cleavage site was made.

The amino acid sequence of this cDNA clone was used in a BLAST homology search. This sequence revealed high homology to the Berberine Bridge Enzymes (BBE) from Californian poppy (*Eschscholtzia californica*) (Dittrich and Kutchan, 1991, Proc. Natl. Acad. Sci. USA 88, 9969-9973) and Opium poppy (*Papaver somniferum*) (Facchini *et al.*, 1996, Plant Physiol. 112, 1669-1677).
BLAST screening of Expressed Sequence Tag (dbEST) databases with the amino acid sequence as shown in SEQ ID NO: 16 revealed homologues of the MS59 protein in *Arabidopsis thaliana* (SEQ ID NO: 21 to SEQ ID NO: 47) and rice (SEQ ID NO: 48).
The EST sequences are listed in the sense orientation considering the orientation of homology to MS59. Sequences of the EST clones were altered by inserting one or two extra unknown nucleotides (N or NN) at frameshift positions in order to obtain one single translation frame with homology to MS59.

EXAMPLE 12

Isolation of the gene encoding WL64 and determination of the nucleotide sequence

Based on the amino acid sequence of the amino-terminus of the WL64 protein (SEQ ID NO: 49, Thr-Ser-Thr-Ser-Ile-Ile-Asp-Arg-Phe-Thr-Gln-(Cys/Ser)-Leu-Asn-Asn-Arg-Ala-Asp-Pro-(Ser)-(Phe)-) a primer (a.a. 1 to 11 of SEQ ID NO: 49) was developed for PCR.

The N-terminal amino acid sequence (SEQ ID NO: 49) revealed high homology to the corresponding portion of the MS59 protein (amino acid residues 20 to 39 in SEQ ID NO: 16).

cDNA was prepared from Poly(A) containing RNA that was isolated from lettuce (*Lactuca sativa* cv. Lollo bionda) leaves that were induced by spraying 5 times with a 10 mM sodium salicylate solution. PCR primers FR-WL64-142 (5'ACT TCT ACT TCT ATT ATT GAT AGG TTT ACT CA3', SEQ ID NO: 52) and MS59 primer 4 (5'AAC TTC TCC IAG IGT IGC ICC IGC TTG IAC CCA3', SEQ ID NO: 3) were used to amplify a 405 bp fragment from the lettuce cDNA pool. PCR products corresponding to this PCR fragment were cloned and sequenced (SEQ ID NO: 53) and revealed an uninterrupted open reading frame (SEQ ID NO: 54).

Table 5. EST sequences showing homology to MS59.
Frameshifts were introduced for optimal aligning of the EST's with the MS59 sequence. In the columns with frameshift 1 and frameshift 2 the position of the frameshift and the shift (frame--->frame) are listed. The (-) mark means, no frameshift present.

SEQ ID NO:	EST name	GenBank accession	Frame (1, 2, 3)	Frameshift 1	Frameshift 2
21	ATTS5925	F19886	2	-	-
22	ATTS0345	Z17771	2	202, 2 ---> 1	-
23	ATTS5268	F14356	2	298, 2 ---> 3	-
24	TC13883	-	1	177, 1 ---> 2	-
25	TC11550	-	2	-	-
26	P_16053	R84094	1	-	-
27	P_22214	N97049	2	310, 2 ---> 3	-
28	P_16873	R90518	3	317, 3 ---> 2	-
29	ATTS2532	Z30784	3	188, 3 ---> 1	312, 1 ---> 2
30	TC11456	-	3	-	-
31	P_8818	T45555	1	98, 1 ---> 3	-
32	P_21340	N96011	1	-	-
33	P_22585	W43206	2	367, 2 ---> 1	-
34	Q_ATTS2533	Z30785	2	-	-
35	P_17333	H76902	2	-	-
36	P_9615	T46352	1	-	-
37	Q_ATTS2959	Z33920	2	-	-
38	P_2730	T20722	1	-	-
39	TC9870	-	2	-	-
40	P_14876	H36354	2	241, 2 ---> 1	-
41	P_21353	N96040	1	89, 1 ---> 2	-
42,43	Q_ATTS3343	Z34583	1	-	-
44	Q_ATTS4954	F14032	2	139, 2 ---> 1	-
45	Q_ATTS1606	Z26512	2	-	-
46	P_7866	T44603	1	222, 1 ---> 3	-
47	AA0410042	24308	2	421, 2 ---> 1	-
48	RICS2381A	D40415	3	-	-

New PCR primers were developed based on the sequence of SEQ ID NO: 53 that is located between the original PCR primers. Primers for 5' RACE: 5'CAC GTT TAT GGA GCG TAA GTT GAA C3' (SEQ ID NO: 55) and for 3' RACE: 5'CAC CCT TCA CAC ATT CAA GCA GC3' (SEQ ID NO: 56) were synthesized and used in 5' and 3' RACE PCR reactions, performed as described in the instructions of the Marathon™ cDNA amplification kit (Clontech laboratories, Inc., Palo Alto, CA). Two partial cDNA clones were amplified by 5' and 3' RACE reactions. Sequence analysis confirmed the identity of the partial cDNA clones which together encode all of the open reading frame including a putative signal peptide and 5' and 3' UTR's (untranslated regions). A full length cDNA clone of 1981 bp (SEQ ID NO: 57) was assembled of which the ORF (pos. 7 to pos. 1629) encodes 540 amino acid residues (SEQ ID NO: 58). The amino terminal signal sequence is represented by the first 27 amino acid residues.

EXAMPLE 13

Characterization and isolation of Berberine Bridge Enzyme genes from *Papaver somniferum* and *Eschscholtzia californica*

Genomic DNA was prepared from leaves of full grown Californian poppy (*Eschscholtzia californica*) and Opium poppy (*Papaver somniferum* cv Marianne) plants.

Primers were designed for the Californian poppy gene (EcBBE) at the start of the mature protein (5' GGT AAT GAT CTC CTT TCT TGT TTG ACC 3', SEQ ID NO: 59) and at the stop codon introducing a *Not* I restriction site just downstream of the TAG stop codon (5' AGA GCG GCC GCT ATA TTA CAA CTT CTC CAC CAT CAC TCC TC 3', SEQ ID NO: 60).

For the Opium poppy gene (PsBBE) primers were designed in a similar way at the start of the presumed mature protein (5' GGT GAT GTT AAT GAT AAT CTC CTC 3', SEQ ID NO: 61) and at the TAG stop codon introducing a *Not* I restriction site (5' AGA GCG GCC GCT ACA ATT CCT TCA ACA TGT AAA TTT CCT C 3', SEQ ID NO: 62).

These primers were used to amplify the mature portion of both the BBE genes.

The PCR products were digested with *Not* I and ligated into vector pET32a (Novagen, Madison, WI) digested with *Eco*R V and *Not* I. The correct insertion of the fragment was confirmed using restriction enzyme analysis and DNA sequencing.

EXAMPLE 14

Characterization and isolation of MS59 homologues from *Arabidopsis thaliana*

In our blast screening we identified 26 EST's with homology to MS59. One EST was found in Rice and the remaining 25 were all found in *A. thaliana*. Homologous EST's were found over the entire length of the MS59 sequence. Analysis of the *Arabidopsis* expressed sequence tags revealed that there are 3 EST's with high homology at the 5' end of the protein (SEQ ID NO: 21,SEQ ID NO: 39 and SEQ ID NO: 40) of which SEQ ID NO: 39 and SEQ ID NO: 40 are overlapping sequences. The 3' part of MS59 showed homology to 7 EST sequences (SEQ ID NO: 24, 27, 32, 34, 41, 43 and 45) of which SEQ ID NO: 24 is overlapping with SEQ ID NO: 43 and SEQ ID NO: 32 is overlapping with SEQ ID NO: 45.

Primers were designed, located at the start of the presumed mature part (possible cleavage sites were predicted according to consensus sequences described by Von Heijne *et al.*, 1983 and Von Heijne, 1985) of the two different EST's homologous with the 5' part of MS59 (SEQ ID NO: 16).
The EST sequence represented by SEQ ID NO: 21 possibly missed the first three amino acid residues of the predicted mature part when compared to the MS59 amino acid sequence (SEQ ID NO:16) and, therefore, A.a. residues 20 to 22 of SEQ ID NO:16 were introduced by including 9 nucleotides at the 5' end of the primer.
Primer located 5' in SEQ ID NO: 21, adding residues 20 to 22 of MS59 (SEQ ID NO: 16): 5' ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC 3' (SEQ ID NO: 63).
Primer located behind the predicted cleavage site of SEQ ID NO: 39 and SEQ ID NO: 40: 5' TCC ATC CAA GAT CAA TTC ATA AAC TGT GTC (SEQ ID NO: 64).

Primers were also made located around the stopcodon of the five different EST's homologous with the 3' part of the MS59 a.a. sequence (SEQ ID NO: 16) and introducing a *Not* I restriction site for cloning in the pET32a *E. coli* expression vector.
Primer located in SEQ ID NO: 24 and SEQ ID NO: 43, 5' AGA GCG GCC GCT TTC ATG AAC CTA GCT TCT AGT AGG 3' (SEQ ID NO: 65). Primer in SEQ ID NO 27, 5' AGA GCG GCC GCG AAA TGG CCC CCC TTT TAA AAC GGG G 3' (SEQ ID

NO: 66). Primer in SEQ ID NO:32 and SEQ ID NO: 41, 5' AGA GCG GCC GCA AAT GAT ATC TTC AGG TAA CTT TGT TCA C (SEQ ID NO: 67). Primer in SEQ ID NO: 34, 5' AGA GCG GCC GCA TAA TCA AAT AAA TAC ACT TAT GGT AAC ACA G (SEQ ID NO: 68) and the primer in SEQ ID NO: 45, 5' AGA GCG GCC GCT GGT TTT GTA TTG AGG ACT CAA AAC AG 3' (SEQ ID NO: 69).

All possible combinations of the 5' primers with the 3' primers were used in a PCR on genomic DNA isolated from *Arabidopsis thaliana* cv Columbia. In a PCR with the primers SEQ ID NO: 63 and SEQ ID NO: 68 an approximately 1800 bp band was amplified. This band was cloned and identity of the PCR product was confirmed by DNA sequencing. The cloned PCR product of 1757 bp (SEQ ID NO: 70) contained an intron from position 570 to position 801, the open reading frame of SEQ ID NO: 70 consists of 508 amino acid residues (SEQ ID NO: 71).

Total RNA was isolated from *Arabidopsis thaliana* Col-0 from 12 days old sterile etiolated seedlings grown in the dark on Murashige and Skoog agar, from 12 days old sterile seedlings grown in liquid Murashige and Skoog medium with a 16 hour photoperiod and from leaves, stems, flowers and siliques from full grown plants (Newman *et al.*, 1994 Plant Physiol. 106: 1241-1255). The RNA from the different developmental stages was pooled. Poly(A)$^+$ RNA was isolated using the Poly(A) Quick® mRNA Isolation kit (Stratagene, La Jolla, CA) and cDNA was prepared using the Marathon™ cDNA Amplification Kit (Clontech Laboratories Inc., Palo Alto, CA).

PCR reactions were set up with the cDNA pool with different combinations of 5' primers and 3' primers. A PCR product was amplified with the primer combination SEQ ID NO: 63 and SEQ ID NO: 68 of approximately 1600 bp. The PCR product was cloned in the *Eco*R V and *Not* I restriction sites of the bacterial expression vector pET32a (Novagen, Madison, WI). The sequence of the PCR product was determined and revealed an uninterrupted open reading frame of 1527 bp (SEQ ID NO: 72) representing a protein of 508 amino acid residues (SEQ ID NO: 73).

A second cDNA clone of about 1600 bp was amplified with the primer combination SEQ ID NO: 64 and SEQ ID NO: 65. This cDNA clone was also ligated into the *Eco*R V and *Not* I restriction sites of pET32a

(Novagen, Madison, WI). This cDNA PCR clone was also characterized by DNA sequencing and consisted of an uninterrupted open reading frame of 1530 bp (SEQ ID NO: 74) encoding 509 amino acid residues (SEQ ID NO: 75).

EXAMPLE 15

Expression of MS59, the Berberine Bridge Enzymes from *Papaver somniferum* and *Eschscholtzia californica* and two homologous proteins from *Arabidopsis thaliana* cv Columbia in *E.coli*

A PCR fragment containing the presumed mature portion of MS59 was introduced in vector pET32c (Novagen, Madison, WI), and the correct insertion of the fragment is confirmed using DNA sequencing. Then, the plasmid was introduced into *E. coli* AD494 (DE3) pLysS (Novagen, Madison, WI). Small scale cultures (2 ml) of several colonies were then started of which half is induced by the addition of IPTG to 1mM final concentration. Total extracts from *E.coli* were run on SDS gels and analyzed by Coomassie Brilliant Blue staining. Several clones exhibited strong overexpression of the MS59 protein. A clone which had strong overexpression was selected for a large scale culture. Five hundred ml of LB supplemented with 0.4 mM glucose was inoculated with a culture of this *E. coli* and grown to an optical density of 0.5-0.7. Then, IPTG was added to a final concentration of 1 mM and protein production allowed for 3 hours at 30°C. A large proportion of the MS59 protein was found in the insoluble protein fraction, a small amount appeared soluble. The resulting insoluble protein preparation contained mainly MS59 protein. This preparation is used for raising antibodies (Example 17). The soluble fraction was used in an *in vitro* assay to test whether the MS59 protein still exhibited antifungal activity.

The pET32a plasmids containing the open reading frames of the four MS59/WL64 homologues were introduced into *E.coli* AD494(DE3)pLysS (Novagen, Madison, WI). Small scale cultures (25 ml) of several independent clones were grown to an optical density of 0.5-0.7. Then IPTG was added to a final concentration of 1mM and protein production was allowed for 4 hours at 30°C.
Soluble and total protein fractions were isolated. The samples were analyzed using SDS-PAGE followed by Neuhoff staining and Western

analysis using the S-Tag Western Blotting detection kit (Novagen, Madison, WI). A large portion of the protein was found in the insoluble fraction, only a small amount appeared to be soluble. Clones which strongly overexpressed the homologous proteins were selected for production of the proteins in large scale cultures of 1.5 liter each.

EXAMPLE 16

In vitro antifungal assays of MS59, MS59/WL64 homologues from Californian poppy (*Eschscholtzia californica*) and Opium poppy (*Papaver somniferum*) and two homologous proteins from *Arabidopsis thaliana*

The MS59 protein produced in *E. coli* contained N-terminal trxA-, His- and S-Tags. The His-tag was used for purification of the soluble MS59 on an IMAC (immobilized metal affinity chromatography) column, charged with Ni^{2+}. Bound protein was eluted by increasing the imidazole concentration. The peak fraction from this purification contains some contaminating *E. coli* proteins.

The peak fraction of this MS59 purification was dialysed into 50 mM MES, pH 6.0, and used in an *in vitro* assay with *Phytophthora infestans* and *Pythium ultimum*. For the standard setup of the in vitro antifungal assay with *Phytophthora infestans* and *Pythium ultimum* see above.

As control treatment we assayed an unrelated His-tagged protein purified from the same expression host, with some *E.coli protein* background. Also a boiled MS59 control (heated 10 minutes at 100°C) was included. Approximately 40 ng of fusion protein was tested in the *Phytophthora infestans* assay, twice that amount was used for the *Pythium ultimum* inhibition assay.

Microtiter dishes were wrapped with Parafilm and incubated in the dark at room temperature. After 2-3 days the mycelium of the growing fungus in the wells was stained with lactophenol cotton blue and the extent of growth was estimated.

IMAC fractions from the soluble fraction of *E.coli* containing MS59 showed complete inhibition of *P.infestans* and *P.ultimum* at concentrations of 20-40 ng.

Table 6. Antifungal effects of MS59 from *E.coli* on *Phytophthora infestans*

Fraction	$MS59_{E. coli}$	$MS59_{E. coli}$ boiled	His-protein E.coli	MES buffer
Growth inhibition	4	0	0	0
amount of extract	5µl	5µl	5µl	

Growth inhibition (GI) is scored visually on a linear scale of 0 (no inhibition) to 4 (complete growth inhibition).

Table 7. Antifungal effects of MS59 from *E.coli* on *Pythium ultimum*

Fraction	$MS59_{E. coli}$	$MS59_{E. coli}$ boiled	His-protein E.coli	MES buffer
Growth inhibition	4	0	0	0
amount of extract	10µl	10µl	10µl	

Microscopical analysis of the wells indicate the rapid germination and subsequent growth of *Phytophthora infestans* zoospores in each of the controls. Germination is near completely inhibited in the reactions containing the MS59 protein from *E. coli*. Some spores do germinate, but hyphal tip growth appears to stop soon after initiation. After 48 hours growth of Phytophthora infestans mycelium is abundant in the controls, but almost undetectable in the assay containing MS59. Even after 72 hours no substantial growth is observed. Fungal hyphae appear somewhat granular and thickened in the reactions containing MS59 protein. Examples of the characteristic patterns of fungal growth in incubations with and without *E. coli*-

produced MS59 are depicted in figure 4. After 48 and 72 hours fungal growth in the control incubations is so extensive no photographic material could be gathered. Incubations in the presence of MS59 leads to complete blockage of further growth, the germination tubes observed at 24 hours do not noticeably extend further.

Likewise, in the *Pythium ultimum* inhibition assay, where mycelium fragments are used, no growth is apparent upon treatment with MS59 (see fig. 5). After 24 hours the control reactions were completely overgrown by mycelium. Only small mycelium fragments are at that stage apparent in the MS59-treated sample.

The poppy homologues were expressed in *E. coli* (pET32a) and tested for *in vitro* antifungal activity on *Phytophtora infestans* and *Pythium ultimum*. *Phytophthora infestans* spores and hyphal fragments of *Pythium ultimum* were suspended in respectively sterile water or potato dextrose broth (PDB). 400-600 spores or 200 fragments/50 µl were added to each well.

The expressed proteins were partially purified by means of IMAC column chromatography. Fractions containing the expressed proteins were buffer exchanged to 50mM MES, pH 6.0, filter sterilized, and tested for their antifungal activity, with IMAC purified *E. coli* pET32-MS59 as a positive control.

No antifungal activity was observed for both *Eschscholtzia californica* and *Papaver somniferum* MS59/WL64 homologues, not even at concentrations ten times higher than that of the positive control, leading to a 100% growth inhibition of both fungi. This could mean that these *E. coli* expressed proteins did not have the correct folding and therefore showed no biological activity.

EXAMPLE 17

Raising antibodies against denatured MS59

Antibodies were raised in rabbits against denatured MS59 (the solubilized form from the insoluble *E.coli* fraction) in PAG-slices. The antibodies showed cross-reaction with a band of about 60 kDa only in the IF of pMOG1180 (Examples 18 and 19) tobacco plants containing antifungal and glucose oxidase activity. Surprisingly, no cross-reaction with WL64 is found.

EXAMPLE 18

Tailoring a MS59 clone for expression in transgenic plants

PCR primers were developed based on the sequence around the ATG start codon and the TGA stop codon for cloning of the open reading frame (ORF). A NcoI restriction site was introduced at the ATG start codon for fusion to a constitutive promoter by PCR using primer: 5' CC GCC ATG GAG ACT TCC ATT CTT ACT C 3' (SEQ ID NO:16). The second codon of the ORF was changed from caa (Q) to gag (E) as a result of the introduced NcoI restriction site.

Downstream of the TGA stop codon a BamHI restriction site was introduced by PCR using primer: 5' GCC GGA TCC TCA AGA TGA CAA AGT TGG GAT GCT 3' (SEQ ID NO:18).

Using a PCR reaction with *Pfu* DNA polymerase, we amplified the entire ORF, using the PCR primers to introduce the NcoI restriction site on the startcodon ATG and the BamHI restriction enzyme recognition site just downstream of the stopcodon. The integrity of the DNA sequence was confirmed by sequencing (SEQ ID NO:19). The entire ORF was linked to a constitutive promoter which allows high level protein expression in most parts of the plant. After the ORF a 3' untranslated region of the potato proteinase inhibitor II (Thornburg *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84, 744-748), which contains sequences needed for polyadenylation (An *et al.*, 1989, Plant Cell 1, 115-122), was introduced. The chimeric gene produced was introduced into binary vector pMOG800 (deposited at the Centraal Bureau voor Schimmelcultures, Baarn, The Netherlands, under CBS 414.93, on August 12, 1993). The resulting clone pMOG1180, which harbours the MS59 construct under control of the ocs-mas hybrid promoter (WO95/14098) was introduced in *Agrobacterium tumefaciens* strain EHA105, suitable for transformation of target crops tomato and potato, strain MOG101 for transformation of tobacco and *Arabidopsis* and MOG301 for transformation of *Brassica napus*.

EXAMPLE 19

Production and analysis of transgenic tobacco and potato plants containing the MS59 gene construct

Using *Agrobacterium* mediated transformation system binary constructs containing the MS59 gene construct as described in Example 18 were introduced into tobacco and potato. The transgenic shoots of these different plant species were regenerated into whole plants and subsequently, primary transformants were analyzed for expression of the newly introduced MS59 gene. For this analysis use was made of Western blotting techniques, using antibodies against MS59 specific peptide coupled to BSA. All antisera were diluted 1:5,000. A concentration series of purified proteins (12.5, 25, 50 and 100 ng) was used to judge the expression level of the introduced proteins in the transgenic plants. Transgenic samples were homogenized in 50 mM sodium acetate buffer pH 5.2 and the extracts were clarified by centrifugation. The supernatants were either directly analyzed or left overnight to precipitate on ice. Overnight precipitation was always followed by clarification (by centrifugation). The protein concentration of the supernatants obtained in either way was determined using Bradford reagent (Bradford 1976, Anal. Biochem. 72, 248-254) and BSA as the standard protein. As much protein as possible (but never more than 10 µg) was loaded on a 12,5 % SDS-PAA gel (Laemmli, supra) and immunoblotted as previously described (Ponstein *et al.* supra).

Extracts from leaves of ms59-transgenic tobacco and potato plants were made by pottering leaf fragments in a buffer containing 50 mM NaAc, (pH = 5.2). After this, insoluble protein was removed by centrifugation. Total soluble protein content was measured and the equivalent of 10 µg was loaded on a SDS-gel. After running the gel the proteins were transferred to blot. This blot was developed using the antiserum raised against purified MS59 (Example 17). The MS59-specific antiserum was used in a 1:5,000 dilution. Purified MS59 was also run alongside on the gel, and is included for reference.

A number of transformed plants selected based on their high level expression of MS59 protein and S1 progeny plants will be tested in fungal infection assays.

EXAMPLE 20

Purification of MS59 transproteins from tobacco transgenics

Transgenic tobacco plants were produced expressing MS59 constitutively. Levels of expression are determined using Western analysis. Extracts of the transgenic material are assayed for *in vitro* growth inhibitory activity against *Phytophthora infestans and Pythium ultimum*. Small scale total extracts were made from *in vitro* leaves of tobacco containing the pMOG1180 construct (mas-ocs-promotor-MS59) and of tobacco control lines. The extracts were made by grinding leaf material in 50 mM NaAc pH 5.2. The supernatant was dialysed against 50 mM MES pH 6.0 and tested for *in vitro* antifungal activity according to the methods described in the general experimental part. Some of the tobacco pMOG1180 lines showed high antifungal activity on *P.infestans* and *P.ultimum* compared to other lines or control lines.

EXAMPLE 21

Carbohydrate oxidase activity / Localization of MS59 in transgenic tobacco

Equal amounts of partial purified soluble MS59 and soluble homologue fractions (*Papaver*, *Eschscholzia*, *Arabidopsis*-A11 and -B7) were tested for carbohydrate oxidase activity. Carbohydrate oxidase activity for MS59 was 0.011 ODu/min and for the homologues 0.0003-0.0012 ODu/min, a difference of a factor 10.

From the transgenic pMOG1180 tobacco lines of Example 20 that showed antifungal activity *in vitro* IF was isolated at a later stage and tested for carbohydrate oxidase activity. Also the material that was left after IF isolation (called "-IF ") was tested. The same lines that showed antifungal activity have high carbohydrate oxidase activity. The activity is located in the IF.

EXAMPLE 22

Introduction of the four genes construct containing Chi-I, Glu-I, AP24 and MS59 under control of a constitutive plant promoter, into tomato, potato, carrot, *Brassica napus* and *Arabidopsis*

Using *Agrobacterium* mediated transformation system binary construct pMOG1145 and pMOG1180 containing the genes encoding Chi-I, Glu-I, AP24 and MS59 or pMOG1146 containing the genes encoding Chi-I, Glu-I, bPR-1 and MS59 is introduced into different crop species including, tomato, potato, carrot, *Brassica napus* and Arabidopsis. S1 progeny plants are tested in fungal infection assays.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: MOGEN International nv
(B) STREET: Einsteinweg 97
(C) CITY: Leiden
(E) COUNTRY: The Netherlands
(F) POSTAL CODE (ZIP): 2333 CB
(G) TELEPHONE: 31-(0)71-5258282
(H) TELEFAX: 31-(0)71-5221471

(ii) TITLE OF INVENTION: Antifungal proteins, DNA coding therefor, and hosts incorporating same.

(iii) NUMBER OF SEQUENCES: 75

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 96.202.466.7
(B) FILING DATE: 04-SEP-1996

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 97.200.831.2
(B) FILING DATE: 10-MAR-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus annuus
(B) STRAIN: cv. zebulon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly
1 5 10 15

Ala Thr Leu Gly Glu Val Tyr Tyr Arg
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus annuus
(B) STRAIN: cv. zebulon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr Pro Gly Xaa Ser
1 5 10 15

Ser Phe Pro Thr Val Leu Gln Asn Tyr
20 25

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AACTTCTCCN AGNGTNGCNC CNGCTTGNAC CCA 33

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: YES

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GATCCNTCTT TCCCNATTAC TGGNGAGGTT TA 32

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus annuus
(B) STRAIN: cv. zebulon

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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1 5 10 15

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TCT TTT CCT ACC GTC TTG CAA AAC TAC ATC CGA AAC CTT CGG TTC AAT     96
Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn
                20                  25                  30

GAA ACT ACC ACA CCA AAA CCC TTT TTA ATC ATC ACA GCC GAA CAT GTT    144
Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr Ala Glu His Val
            35                  40                  45

TCC CAC ATT CAG GCA GCT GTG GTT TGT GGC AAA CAA AAC CGG TTG CTA    192
Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln Asn Arg Leu Leu
        50                  55                  60

CTG AAA ACC AGA AGC GGT GGT CAT GAT TAT GAA GGT CTT TCC TAC CTT    240
Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu
65                  70                  75                  80

ACA AAC ACA AAC CAA CCC TTC TTC ATT GTG GAC ATG TTC AAT TTA AGG    288
Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met Phe Asn Leu Arg
                85                  90                  95

TCC ATA AAC GTA GAT ATC GAA CAA GAA ACC GCA TGG GTC CAA GCC GGC    336
Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly
            100                 105                 110

GCC ACC CTC GGA GAA GTT                                            354
Ala Thr Leu Gly Glu Val
        115
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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn
            20                  25                  30

Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr Ala Glu His Val
        35                  40                  45

Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln Asn Arg Leu Leu
    50                  55                  60

Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu
65                  70                  75                  80
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Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met Phe Asn Leu Arg
85 90 95

Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp Val Gln Ala Gly
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Ala Thr Leu Gly Glu Val
115

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CAGGCAGCTG TGGTTTGTGG C 21

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTCCACAATG AAGAAGGGTT G 21

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1
(D) OTHER INFORMATION: /function= "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACGTAGATAT CGAACAAGAA ACCGC 25

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GCTTTACTAC ACGGGCTTCC CCAG 24

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CTGGGGAAGC CCGTGTAGTA AAGC 24

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGTACTCCAA CCACGGCGCT C 21

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CGGGAAGTTG CAGAAGATTG GGTTG 25

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GAGCAAGAGA AGAAGGAGAC 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1784 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus annuus
(B) STRAIN: Zebulon

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 21..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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                      Met Gln Thr Ser Ile Leu Thr Leu Leu Leu
                        1               5                  10

CTC TTG CTC TCA ACC CAA TCT TCT GCA ACT TCC CGT TCC ATT ACA GAT    98
Leu Leu Leu Ser Thr Gln Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp
                15                  20                  25

CGC TTC ATT CAA TGT TTA CAC GAC CGG GCC GAC CCT TCA TTT CCG ATA   146
Arg Phe Ile Gln Cys Leu His Asp Arg Ala Asp Pro Ser Phe Pro Ile
            30                  35                  40

ACC GGA GAG GTT TAC ACT CCC GGA AAC TCA TCT TTT CCT ACC GTC TTG   194
Thr Gly Glu Val Tyr Thr Pro Gly Asn Ser Ser Phe Pro Thr Val Leu
        45                  50                  55

CAA AAC TAC ATC CGA AAC CTT CGG TTC AAT GAA ACT ACC ACA CCA AAA   242
Gln Asn Tyr Ile Arg Asn Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys
    60                  65                  70

CCC TTT TTA ATC ATC ACA GCC GAA CAT GTT TCC CAC ATT CAG GCA GCT   290
Pro Phe Leu Ile Ile Thr Ala Glu His Val Ser His Ile Gln Ala Ala
75                  80                  85                  90

GTG GTT TGT GGC AAA CAA AAC CGG TTG CTA CTG AAA ACC AGA AGC GGT   338
Val Val Cys Gly Lys Gln Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly
                95                  100                 105
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GGT CAT GAT TAT GAA GGT CTT TCC TAC CTT ACA AAC ACA AAC CAA CCC   386
Gly His Asp Tyr Glu Gly Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro
            110                 115                 120

TTC TTC ATT GTG GAC ATG TTC AAT TTA AGG TCC ATA AAC GTA GAT ATC   434
Phe Phe Ile Val Asp Met Phe Asn Leu Arg Ser Ile Asn Val Asp Ile
        125                 130                 135

GAA CAA GAA ACC GCA TGG GTC CAA GCC GGT GCG ACT CTT GGT GAA GTG   482
Glu Gln Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu Gly Glu Val
    140                 145                 150

TAC TAT CGA ATA GCG GAG AAA AGT AAC AAG CAT GGT TTT CCG GCA GGG   530
Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Lys His Gly Phe Pro Ala Gly
155                 160                 165                 170

GTT TGT CCA ACG GTT GGC GTT GGT GGG CAT TTT AGT GGT GGT GGG TAT   578
Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly Gly Gly Tyr
                175                 180                 185

GGT AAT TTG ATG AGA AAA TAT GGT TTG TCG GTT GAT AAT ATT GTT GAT   626
Gly Asn Leu Met Arg Lys Tyr Gly Leu Ser Val Asp Asn Ile Val Asp
            190                 195                 200

GCT CAA ATA ATA GAT GTG AAT GGC AAG CTT TTG GAT CGA AAG AGT ATG   674
Ala Gln Ile Ile Asp Val Asn Gly Lys Leu Leu Asp Arg Lys Ser Met
        205                 210                 215

GGT GAG GAT TTG TTT TGG GCG ATC ACC GGC GGT GGT GGT GTT AGT TTT   722
Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly Val Ser Phe
    220                 225                 230

GGT GTG GTT CTA GCC TAC AAA ATC AAA CTA GTT CGT GTT CCG GAG GTT   770
Gly Val Val Leu Ala Tyr Lys Ile Lys Leu Val Arg Val Pro Glu Val
235                 240                 245                 250

GTG ACC GTG TTT ACC ATT GAA AGA AGA GAG GAA CAA AAC CTC AGC ACC   818
Val Thr Val Phe Thr Ile Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr
                255                 260                 265

ATC GCG GAA CGA TGG GTA CAA GTT GCT GAT AAG CTA GAT AGA GAT CTT   866
Ile Ala Glu Arg Trp Val Gln Val Ala Asp Lys Leu Asp Arg Asp Leu
            270                 275                 280

TTC CTT CGA ATG ACC TTT AGT GTC ATA AAC GAT ACC AAC GGT GGA AAG   914
Phe Leu Arg Met Thr Phe Ser Val Ile Asn Asp Thr Asn Gly Gly Lys
        285                 290                 295

ACA GTC CGT GCT ATC TTT CCA ACG TTG TAC CTT GGA AAC TCG AGG AAT   962
Thr Val Arg Ala Ile Phe Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn
    300                 305                 310

CTT GTT ACA CTT TTG AAT AAA GAT TTC CCC GAG TTA GGG TTG CAA GAA  1010
Leu Val Thr Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu
315                 320                 325                 330
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TCG GAT TGT ACT GAA ATG AGT TGG GTT GAG TCT GTG CTT TAC TAC ACG  1058
Ser Asp Cys Thr Glu Met Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr
                335                 340                 345

GGC TTC CCC AGT GGT ACT CCA ACC ACG GCG CTC TTA AGC CGT ACT CCT  1106
Gly Phe Pro Ser Gly Thr Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro
            350                 355                 360

CAA AGA CTC AAC CCA TTC AAG ATC AAA TCC GAT TAT GTG CAA AAT CCT  1154
Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro
        365                 370                 375

ATT TCT AAA CGA CAG TTC GAG TTC ATC TTC GAA AGG CTG AAA GAA CTT  1202
Ile Ser Lys Arg Gln Phe Glu Phe Ile Phe Glu Arg Leu Lys Glu Leu
    380                 385                 390

GAA AAC CAA ATG TTG GCT TTC AAC CCA TAT GGT GGT AGA ATG AGT GAA  1250
Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu
395                 400                 405                 410

ATA TCC GAA TTC GCA AAG CCT TTC CCA CAT AGA TCG GGT AAC ATA GCG  1298
Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly Asn Ile Ala
                415                 420                 425

AAA ATT CAA TAC GAA GTA AAC TGG GAG GAT CTT AGC GAT GAA GCC GAA  1346
Lys Ile Gln Tyr Glu Val Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu
            430                 435                 440

AAT CGT TAC TTG AAT TTC ACA AGG CTG ATG TAT GAT TAC ATG ACC CCA  1394
Asn Arg Tyr Leu Asn Phe Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro
        445                 450                 455

TTT GTG TCG AAA AAC CCT AGA AAA GCA TTT TTG AAC TAT AGG GAT TTG  1442
Phe Val Ser Lys Asn Pro Arg Lys Ala Phe Leu Asn Tyr Arg Asp Leu
    460                 465                 470

GAT ATT GGT ATC AAC AGC CAT GGC AGG AAT GCT TAT ACT GAA GGA ATG  1490
Asp Ile Gly Ile Asn Ser His Gly Arg Asn Ala Tyr Thr Glu Gly Met
475                 480                 485                 490

GTT TAT GGG CAC AAG TAT TTC AAA GAG ACA AAT TAC AAG AGG CTA GTA  1538
Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val
                495                 500                 505

AGT GTG AAG ACT AAA GTT GAT CCT GAC AAC TTC TTT AGG AAT GAG CAA  1586
Ser Val Lys Thr Lys Val Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln
            510                 515                 520

AGC ATC CCA ACT TTG TCA TCT T GAAGAACGTA CATATATAAA TAAATACCTT   1638
Ser Ile Pro Thr Leu Ser Ser
        525

TGTGCATGGT ATTTTCAGGG TGTTAAAGTG ATATTCAGAT ATTTATGATA GAATTTTGAC 1698

TTGTATTTTA TACAATCAAA ATTGTATGGT TCTCCGAATT TCTCTTTTTA ATTCTGAAAA 1758
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ATACATATTA GTATTGTCAA AAAAAA 1784

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 529 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Gln Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Ser Thr Gln
1 5 10 15

Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
20 25 30

His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
35 40 45

Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
50 55 60

Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr
65 70 75 80

Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln
85 90 95

Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly
100 105 110

Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met
115 120 125

Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp
130 135 140

Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu
145 150 155 160

Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly
165 170 175

Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys
180 185 190

Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val
195 200 205

Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp
210 215 220

Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr
225 230 235 240

Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile
245 250 255

Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val
260 265 270

Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe
275 280 285

Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe
290 295 300

Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn
305 310 315 320

Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met
325 330 335

Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr
340 345 350

Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe
355 360 365

Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe
370 375 380

Glu Phe Ile Phe Glu Arg Leu Lys Glu Leu Glu Asn Gln Met Leu Ala
385 390 395 400

Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys
405 410 415

Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val
420 425 430

Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe
435 440 445

Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro
450 455 460

Arg Lys Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser
465 470 475 480

His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr
485 490 495

Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val
500 505 510

Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser
515 520 525

Ser

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CCGCCATGGA GACTTCCATT CTTACTC 27

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GCCGGATCCT CAAGATGACA AAGTTGGGAT GCT 33

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1590 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Helianthus annuus
(B) STRAIN: Zebulon

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1590

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
ATG GAG ACT TCC ATT CTT ACT CTC CTT CTT CTC TTG CTC TCA ACC CAA      48
Met Glu Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Ser Thr Gln
 1               5                  10                  15

TCT TCT GCA ACT TCC CGT TCC ATT ACA GAT CGC TTC ATT CAA TGT TTA      96
Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
            20                  25                  30

CAC GAC CGG GCC GAC CCT TCA TTT CCG ATA ACC GGA GAG GTT TAC ACT     144
His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
        35                  40                  45

CCC GGA AAC TCA TCT TTT CCT ACC GTC TTG CAA AAC TAC ATC CGA AAC     192
Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
    50                  55                  60

CTT CGG TTC AAT GAA ACT ACC ACA CCA AAA CCC TTT TTA ATC ATC ACA     240
Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr
65                  70                  75                  80

GCC GAA CAT GTT TCC CAC ATT CAG GCA GCT GTG GTT TGT GGC AAA CAA     288
Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln
                85                  90                  95

AAC CGG TTG CTA CTG AAA ACC AGA AGC GGT GGT CAT GAT TAT GAA GGT     336
Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly
            100                 105                 110

CTT TCC TAC CTT ACA AAC ACA AAC CAA CCC TTC TTC ATT GTG GAC ATG     384
Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met
        115                 120                 125

TTC AAT TTA AGG TCC ATA AAC GTA GAT ATC GAA CAA GAA ACC GCA TGG     432
Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp
    130                 135                 140

GTC CAA GCC GGT GCG ACT CTT GGT GAA GTG TAC TAT CGA ATA GCG GAG     480
Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu
145                 150                 155                 160

AAA AGT AAC AAG CAT GGT TTT CCG GCA GGG GTT TGT CCA ACG GTT GGC     528
Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly
                165                 170                 175
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GTT GGT GGG CAT TTT AGT GGT GGT GGG TAT GGT AAT TTG ATG AGA AAA   576
Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys
            180                 185                 190

TAT GGT TTG TCG GTT GAT AAT ATT GTT GAT GCT CAA ATA ATA GAT GTG   624
Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val
        195                 200                 205

AAT GGC AAG CTT TTG GAT CGA AAG AGT ATG GGT GAG GAT TTG TTT TGG   672
Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp
    210                 215                 220

GCG ATC ACC GGC GGT GGT GGT GTT AGT TTT GGT GTG GTT CTA GCC TAC   720
Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr
225                 230                 235                 240

AAA ATC AAA CTA GTT CGT GTT CCG GAG GTT GTG ACC GTG TTT ACC ATT   768
Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile
                245                 250                 255

GAA AGA AGA GAG GAA CAA AAC CTC AGC ACC ATC GCG GAA CGA TGG GTA   816
Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val
            260                 265                 270

CAA GTT GCT GAT AAG CTA GAT AGA GAT CTT TTC CTT CGA ATG ACC TTT   864
Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe
        275                 280                 285

AGT GTC ATA AAC GAT ACC AAC GGT GGA AAG ACA GTC CGT GCT ATC TTT   912
Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe
    290                 295                 300

CCA ACG TTG TAC CTT GGA AAC TCG AGG AAT CTT GTT ACA CTT TTG AAT   960
Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn
305                 310                 315                 320

AAA GAT TTC CCC GAG TTA GGG TTG CAA GAA TCG GAT TGT ACT GAA ATG  1008
Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met
                325                 330                 335

AGT TGG GTT GAG TCT GTG CTT TAC TAC ACG GGC TTC CCC AGT GGT ACT  1056
Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr
            340                 345                 350

CCA ACC ACG GCG CTC TTA AGC CGT ACT CCT CAA AGA CTC AAC CCA TTC  1104
Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe
        355                 360                 365

AAG ATC AAA TCC GAT TAT GTG CAA AAT CCT ATT TCT AAA CGA CAG TTC  1152
Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe
    370                 375                 380

GAG TTC ATC TTC GAA AGG ATG AAA GAA CTT GAA AAC CAA ATG TTG GCG  1200
Glu Phe Ile Phe Glu Arg Met Lys Glu Leu Glu Asn Gln Met Leu Ala
385                 390                 395                 400
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TTC AAC CCA TAT GGT GGT AGA ATG AGT GAA ATA TCC GAA TTC GCA AAG  1248
Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys
                405                 410                 415

CCT TTC CCA CAT AGA TCG GGT AAC ATA GCG AAG ATT CAA TAC GAA GTA  1296
Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val
            420                 425                 430

AAC TGG GAG GAT CTT AGC GAT GAA GCC GAA AAT CGT TAC TTG AAT TTC  1344
Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe
        435                 440                 445

ACA AGG CTG ATG TAT GAT TAC ATG ACT CCA TTT GTG TCG AAA AAC CCT  1392
Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro
    450                 455                 460

AGA GAA GCA TTT TTG AAC TAT AGG GAT TTG GAT ATT GGT ATC AAC AGC  1440
Arg Glu Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser
465                 470                 475                 480

CAT GGC AGG AAT GCT TAT ACT GAA GGA ATG GTT TAT GGG CAC AAA TAT  1488
His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr
                485                 490                 495

TTC AAA GAG ACA AAT TAC AAG AGG CTA GTA AGT GTG AAG ACT AAA GTT  1536
Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val
            500                 505                 510

GAT CCT GAC AAC TTC TTT AGG AAT GAG CAA AGC ATC CCA ACT TGG TCA  1584
Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser
        515                 520                 525

TCT TG                                                          1590
Ser
    530
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 529 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
Met Glu Thr Ser Ile Leu Thr Leu Leu Leu Leu Leu Leu Ser Thr Gln
  1               5                  10                  15

Ser Ser Ala Thr Ser Arg Ser Ile Thr Asp Arg Phe Ile Gln Cys Leu
            20                  25                  30

His Asp Arg Ala Asp Pro Ser Phe Pro Ile Thr Gly Glu Val Tyr Thr
        35                  40                  45
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Pro Gly Asn Ser Ser Phe Pro Thr Val Leu Gln Asn Tyr Ile Arg Asn
     50                  55                  60

Leu Arg Phe Asn Glu Thr Thr Thr Pro Lys Pro Phe Leu Ile Ile Thr
 65                  70                  75                  80

Ala Glu His Val Ser His Ile Gln Ala Ala Val Val Cys Gly Lys Gln
                 85                  90                  95

Asn Arg Leu Leu Leu Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly
            100                 105                 110

Leu Ser Tyr Leu Thr Asn Thr Asn Gln Pro Phe Phe Ile Val Asp Met
        115                 120                 125

Phe Asn Leu Arg Ser Ile Asn Val Asp Ile Glu Gln Glu Thr Ala Trp
    130                 135                 140

Val Gln Ala Gly Ala Thr Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu
145                 150                 155                 160

Lys Ser Asn Lys His Gly Phe Pro Ala Gly Val Cys Pro Thr Val Gly
                165                 170                 175

Val Gly Gly His Phe Ser Gly Gly Gly Tyr Gly Asn Leu Met Arg Lys
            180                 185                 190

Tyr Gly Leu Ser Val Asp Asn Ile Val Asp Ala Gln Ile Ile Asp Val
        195                 200                 205

Asn Gly Lys Leu Leu Asp Arg Lys Ser Met Gly Glu Asp Leu Phe Trp
    210                 215                 220

Ala Ile Thr Gly Gly Gly Gly Val Ser Phe Gly Val Val Leu Ala Tyr
225                 230                 235                 240

Lys Ile Lys Leu Val Arg Val Pro Glu Val Val Thr Val Phe Thr Ile
                245                 250                 255

Glu Arg Arg Glu Glu Gln Asn Leu Ser Thr Ile Ala Glu Arg Trp Val
            260                 265                 270

Gln Val Ala Asp Lys Leu Asp Arg Asp Leu Phe Leu Arg Met Thr Phe
        275                 280                 285

Ser Val Ile Asn Asp Thr Asn Gly Gly Lys Thr Val Arg Ala Ile Phe
    290                 295                 300

Pro Thr Leu Tyr Leu Gly Asn Ser Arg Asn Leu Val Thr Leu Leu Asn
305                 310                 315                 320

Lys Asp Phe Pro Glu Leu Gly Leu Gln Glu Ser Asp Cys Thr Glu Met
                325                 330                 335
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Ser Trp Val Glu Ser Val Leu Tyr Tyr Thr Gly Phe Pro Ser Gly Thr
340 345 350

Pro Thr Thr Ala Leu Leu Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe
355 360 365

Lys Ile Lys Ser Asp Tyr Val Gln Asn Pro Ile Ser Lys Arg Gln Phe
370 375 380

Glu Phe Ile Phe Glu Arg Met Lys Glu Leu Glu Asn Gln Met Leu Ala
385 390 395 400

Phe Asn Pro Tyr Gly Gly Arg Met Ser Glu Ile Ser Glu Phe Ala Lys
405 410 415

Pro Phe Pro His Arg Ser Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val
420 425 430

Asn Trp Glu Asp Leu Ser Asp Glu Ala Glu Asn Arg Tyr Leu Asn Phe
435 440 445

Thr Arg Leu Met Tyr Asp Tyr Met Thr Pro Phe Val Ser Lys Asn Pro
450 455 460

Arg Glu Ala Phe Leu Asn Tyr Arg Asp Leu Asp Ile Gly Ile Asn Ser
465 470 475 480

His Gly Arg Asn Ala Tyr Thr Glu Gly Met Val Tyr Gly His Lys Tyr
485 490 495

Phe Lys Glu Thr Asn Tyr Lys Arg Leu Val Ser Val Lys Thr Lys Val
500 505 510

Asp Pro Asp Asn Phe Phe Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser
515 520 525

Ser

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..350

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAGAAACTCG GAGACTTTCA CACAATGCCT AACCTCAAAC TCCGACCCCA AACATCCCAT 60

CTCCCCCGCT ATCTTCTTCT CCGGAAATGG CTCCTACTCC TCCGTATTAC AAGCCAACAT 120

CCGTAACCTC CGCTTCAACA CCACCTCAAC TCCGAAACCC TTCCTCATAA TCGCCGCAAC 180

ACATGAATCC CATGTGCAAG CCGCGATTAC TTGCGGGAAA CGCCACAACC TTCAGATGAA 240

AATCAGAAGT GGAGGCCACG ACTACGATGG CTTGTCATAC GTTACATACT CTGGCAAACC 300

GTTCTTCGTC CTCGACATGT TTAACCTCCG TTCGGTGGAT GTCGACGTGG 350

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 278 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..278

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGCATGGATC TCCGCCGGAG CGACTCTCGG AGAGGTTTAT TATCGGATTT GGGAGAAAAG 60

CAGAGTCCAT GGATTCCCCG CCGGAGTTTG ACCGACGGTT GGTGTTGGTG GGCATTTAAG 120

CGGCGGTGGT TACGGTAACA TGGTGAGGAA GTTTGGATTA TCTGTGGATT ACGTTGAGGA 180

TGCCAAGATC GTCGATGTAA ACNGTCGGGT TTTAGATCGG AAAGCAATGG GTGAGGATCT 240

GTTCTGGGCG ATTACCGGTG GAGGAGGAGG TAGCGTAC 278

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 345 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..345

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TGGACATATT AGCGGAGGAG GATTCGGTAC AATAATGAGG AAATACCCTT TAGCGTCTGA 60

TAACGTTGTG GACGCACGTT TGATGGATGT AAATGGGAAA ACTCTTGACC GGAAAACGAT 120

GGGAGAGGAT TTGTTTTGGG CGCTTAGAGG CGGTGGAGCT GCGAGTTTTG GCGTTGTCTT 180

GTCGTGGAAG GTTAAGCTTG CTAGGGTTCC TGAAAAGGTA ACTTGTTTCA TAAGTCAACA 240

TCCGATGGGA CCTAGCATGA ACAAGCTTGT TCATAGATGG CAATCCATAG GATCAAGANN 300

GCTAGACGAA GATTTATTCA TCAGAGTCAA TATTGACAAC AGTCT 345

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 695 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..695

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GTTCGTTAAA ACCTATCCTN NANGGGCNAA AGNATATCAA AGNTTGNTTA NGNAACCCAA 60

NATTTCTGAA CTGGCCNCCT TCGGTGGTAT ATGNCNAAAN CCCTTGAATC TGCGNANCCN 120

ATTCCGCATA GAAACGGAAC CCTCTTCAAG ATTCTCTATT TACNCGAACT GNCTAGANNG 180

AATGACAAGA CATCGAGTAG NAAAATCAAC TGGATCAAAG AGATATACAA TTACATGGCG 240

CCTTATGTCT CAAGCAATCC AAGACAAGCA TATGTGAACT ACAGAGATCT AGACTTCGGA 300

CAGAACAAGA ACAACGCAAA GGTTAACTTC ATTGAAGCTA AAATCTGGGG ACCTAAGTAC 360

TTCAAAGGCA ATTTTGACAG ATTGGTGAAG ATTAAAACCA AGGTTGATCC AGAGAACTTC 420

TTCAGGCACG AGCAGAGTAT CCCACCTATG CCCTACTAGA AGCTAGGTTC ATGAAACCAA 480

TAACATTATC AAAAATAAGR ATAAATGRTA ATTGTATACA ACATGATTCG KCTTTCTTTA 540

TTTCAGACAA TGTGGACACT ACTCTAAANT AAAAWGTCNA TTTACCTTAA AAAAAAAATA 600

ATCCCCNNTA ANANAAAANT GGGGGGGCCN TTTTTGGGGN TCCCGGTTTT NGGACGGGGN 660

GCTTTNGGGG GGCTTGGNNT TTTTTTNGGN GCCCC 695

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 495 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..495

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

TCTGTTTTNA GGCAGAGCAG AGGAAGTTGT TGCTTTGCTT GGTAAGGAGT TTCCTGAATT 60

NAGTTTAAAG AAGGAGAACT GTTCGGAGAT GACTTGGTTT CAGTCAGCTT TATGGTGGGA 120

TAATCGTGTT AACCCTACTC ANATTGATCC WAAAGTGTTT CTCGATCGGA ATCTTGATAG 180

AGCGAATTTC GGAAAGAGGA AATCGGATTA CGTTGCGAGT AAGATTCCTA GAGATGGGAT 240

TAAGYCTTTT TCCAAGARGA TGMCTGACCT GGGGAAAAYC GGGCTTGTTT TTAAWCCGTA 300

TGGTGGGAAA ATGGCGGAGG TTACGGTTAA CGCGACGCCG TTTCCNCACC GAAGCAAGCT 360

TTTTAAGATT CAGTACTCGG TGACTTNGCA AGAAAACTCT NTCGAGATAG AGAAAGGGTT 420

TCTTGAATCA GGCTAACGTC CTTATAGGTT CATGACCGGG TTTTTNAGCA AGANCCCTGG 480

AATNCTTACT TNAAT 495

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 204 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

AAATTAAAAC AAATCAATGT TGATATTGAA TCCAATAGTG CTTGGTTTCA ACCTGGTGCT 60

ACGCTTGGTG AGCTTTACTA CAGAATTNCA GAGAAGAGCA AAATCCATGG ATTTCCNGCG 120

GGTTTNTNCA CAAGCNTAGG CATAGGTGGG TATATNANAG GCGGTGGATA CGGTACCTTG 180

ATGAGGAAGT ATGGTCTTNC GGGA 204

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..491

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAGATTTCTC GAGCAAGATA CTCCACTGAT GATCTTTGAG CCATTGGGTG GGAAAATCAG 60

CAAGATTTCA GAAACAGAAT CTCCATATCC ACACAGAAGA GGTAATCTGT ATAATATACA 120

GTACATGGTG AAATGGAAAG TGAATGANGT CGAGGAGATG AACAAACATG TCAGGTGGAT 180

GAGATCGTTA CACGATTACA TGACTCCGTA TGTTTCTAAA TCGCCGAGAG GAGCTTATTT 240

GANTTACAGA GATCTTGATT TGGGCTCGAC CAAAGGGATT AACACGGGTT TCGGAGATGC 300

AAGGAAATGG NNGGGTGAGN CTTTTTTCAA AGGTAATTTC CAAGGGGTTA GGTTTTGGTT 360

AAAGGGGAGG TTTNNCCCAN CAAATTTTTT TTCAGGANCC GGCCANGNTT TTCCCCCCCC 420

TNTTTTTNGG NCCCCAATCN AAANCCCCGT TTTAAAAGGG GGGCCATTTC NTTTTTTNCA 480

NNTTAAAAGG G 491

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 407 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..407

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

ATTTGTTCGT GAGGTTAACT TTGACTTTAG TCAACGGTAC GAAGCCTGGT GAGAATACGG 60

TTTTAGCGAC TTTCATTGGG ATGTATTTAG GCCGGTCGGA TAAGCTGTTG ACCGTNATGA 120

ACCGGGATTT CCCGGAGTTG AAGCTGAAGA AAACCGATTN TACCGAGATG AGATGGATCG 180

ATTCGGTTCT GTTTTGGGAC GATTATCCGG TTGGTACACC GACTTCTGTG CTACTAAATC 240

CGCTAGTCGC AAAAAAGTTG TTCATGAAAC GAAAATCGGA CTACGTGAAG CGTCTNATTT 300

TCGAGAACCC GATCTCNNGT TTGATACTCA AGAAATTTGT AGAGGTTNNG AAAGTTAAAA 360

TNAATTTGGA TCCGCATTNN GGNANNNATG GTGAAACCCC NNGTTNT 407

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ACGGCGTCGT ATTGGCCTAC AAAATAAACC TTGTTGAAGT CCCAGAAAAC GTCACCGTTT 60

TCAGAATCTC CCGGACGTTA GAACAAAATG CGACGGATAT CATTCACCGG TGGCAACAAG 120

TTGCACCGAA GCTTCCCGAC GAGCTTTTCA TAAGANCAGT CATTGACGTA NAAACGGCAC 180

TGTTTCATNN CTCAAAAGAC CGTCAGACAA CATTCATAGC AATGTTTCTA GGAGACACGN 240

CAACTCTACT GTCGATATTA AACCGGAGAT TCCCAGAATT GGGTTTGGTC CGGTCTGACT 300

GTACCGNAAC AAGCNNTTGG ATCCAATCTG TGCTATTTTT GGGACAAATA TCCCAGGTTG 360

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 427 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..427

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

TCTTCACTGT CACCAAAACG TTAGAACAAG ACGCAAGATT GAAGACTATT TCTAAGTGGC 60

AACAAATTTC ATCCAAGATT ATTGAAGAGA TACACATCCG AGTGGTACTC AGAGCAGCTG 120

GAAATGATGG AAACAAGACT GTGACAATGA CCTACCTAGG TCAGTTTCTT GGCGAGAAAG 180

GCACCTTGCT GAAGGTTATG GAGAAGGCTT TTCCAGAACT AGGGTTAACT CAAAAGGATT 240

GTACTGAAAT GAGCTGGATT GAAGCCGCCC TTTTCCATGG TGGRTTTCCA ACAGGKTCTC 300

CTATTGAAAT TTTGCTTMAG CTCAAGTCGC CTYTAGGAAA AGRTTWCTTC AAAGCAACGK 360

CGGATTTCGT TAAAGAACCT WTTCCTGTGA TAGGGCTCAA AGGAATATTC AAAAGATTGA 420

TTGAAGG 427

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 437 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..437

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GTTGTACTAT CATNGAAGAT TAAGTTAGTC GATGTTCCGT CCACGGTCAC CGNGTTTAAA 60

GTCCAGAAAC ATNAGGAGAA AGAGGCCGTT AGGNTCATCA ACAAGTGGCA GTATGTTGCG 120

GATAAGGTCC CTGAAGATCT TTTCATCAGC GCAACGTTGG NGAGATCAAA CGGAAACTCT 180

GTGCAGGCTT TGTTTACTGG ACTCTATCTT GGNCCGGTGA ATAATNTCTT GGCCTTGATG 240

GAAGAAAAGT TTCCAGANTT AGGTCTTGAT ATCCAAGNCT GCACAGAGAT GAGTTGGGCT 300

GAATCTGCAC TCTGGTNTNC TGNTTTCNCT AAAGGAGAGN CTCCTTGGGT GTTCCNCGCG 360

GATCGGNAGC GGNCAATTTN TGGNCTTTCA AGGGGAAAGN CGGCTTTTTN CAAGAACCCG 420

NTACCCGGGG TTCAATT 437

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 441 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..441

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

GCGGACCCTA TAGATCANNA TGTGCTACTG ANAGAAGAGG AAGCCAAGAA CAAGCCGGAG 60

ACAGATAAAT ATCTGAAATG GGNCGATANC GTTTACGAAT TTATGACNCC ATATGTTTCG 120

AAATCTCCAA GAGGAGCTTA TGTCAATTTC AAGGATATGG ATTTGGGTAT GTATCTTGGA 180

AAGAAGAAGA CAAAGTACGA GGAAGGAAAG AGTTGGGGAG TGAAGTATTT CAAGAACAAT 240

TTCGAGAGAT TGGTGAGAGT GAAGACTAGG GTTGATCCAA CAGATTTCTT CTGCGATGAA 300

CAGAGCATTC CTCTGGTGAA CAAAGTTACC TGAAGATATC ATTTGAAGTT TTTTATTAGT 360

CCCTTTTCTC TGTGAAATCA TCTGTGCGTG TTGAATATTA TGCGTCAAGT GTGTAACTTA 420

TGTGTGTGAT TGTGAATTGT G 441

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 502 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..502

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTGGCTTAAC ACAACGTCGT TTTGGGCCAA TTACCCGGCG GGTACACCCA AGAGCATCCT 60

TCTAGATAGG CCTCCGACGA ATTCAGTGTC ATTTAAGAGT AAATCGGATT TTGTCAAAAA 120

ACCAATACCC AAAAAAGGTT TAGAGAAGCT TTGGAAGACA ATGTTTAAAT TCAACAGTAG 180

CGTCTCGTTG CAATTCAACC CTTACGGTGG AGTGATGGAC CGGATTCCGG CAACGGCCAC 240

CGCTTTTCCT CATCGGAAAG GAAACTTGTT CAAGGTTCAA TACNCTACGA TGTGGTTTGA 300

CGCAAACGCC ACACAGAGTA GCCNGGCTAT GATGAATGAG CTTTTTGAGG TGGCGGGACC 360

GTACGTGNGT CAAGTAAACC CGAGANANGG CTTCCTTTAA NTTCAGAGNC CATCGNTNTT 420

NGGAGCAANN CCAAGTGGGG GGGNCCAACC GGGGGNTNAA ANCNNAGNTC TTNGGGGGCC 480

CAGAATTTCC TTNGGGGAAT TT 502

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..400

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

NGGGAATTGC NCGAGGNAAG TTGTACCCAA TTCCTGGACC ACCATTGGTT TCCCAAGAAN 60

CCCGAGACAA CCGTTTTTCA ATNACCGTGA TGTTGATTTG GGTATTAATT CTCATAATGG 120

TAAAATCAGT AGTTATGTGG AAGGTAAACG TTACGGGAAG AAGTATTTCG CAGGTAATTT 180

CGAGAGATTG GTGAAGATTA AGACGAGAGT TGATAGTGGT AATTTCTTTA GGAACGAACA 240

GAGTATTCCT GTGTTACCAT AAGTGTATTT ATTTGATTAT TGGTTAGTGA AATTTGTTGT 300

TGTATAATGA TTATATGTCG TATTTTTATT TATTATTAGT AATTTATAAA GTTTGATATT 360

AAATACAAAT AGTATAATAA GATAGTTTCT TTTAGTAAAA 400

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 383 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..383

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CAACTCTAAT GGGAACACCT ACTTCGATCG AATGTCGATG GGGGAAGAGC TTTTCTGGGC 60

GGTTCGAGGA GGTGGAGCCG CGAGTTTCGG CATCGTGATG GGATACAAAA TCCGGTTGGT 120

TCCGGTTCCG GAGAAAGTTA CGGTTTTTAG CGTCGGAAAA ACCGTCGGAG AAGGAGCCGT 180

TGATCTTATA ATGAAGTGGC AGAACTTCTC TCATAGTACG GNTCGGAATT TNTTTGTGAA 240

GCTGANTTTT GANTTTAGTC AACGGTGCAA AGCCGGGTGA AAAAAAGGTT TTAGNGNCTT 300

TCANTTTGGN TGNAANCTTG GGGGTTTTAT NAGAACGGTT AACCGGGATT NANCCCGNGT 360

TTTCCCGGGG TTAAAACCTT NGG 383

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

ATCAATGTTC TTACTAAACG TACACGAGCA TCGTTGGCTT TCAAGGCTAA ATCTGATTTT 60

NTTCAAGAAC CGATNCCTAA AACCGCGATT TCGAAGCTTT GGAGACGGTT GCAAGAACCG 120

GAAGCAGAGC ATGCTCAGCT AATTTNCACN CCATTTGGTG GTAAAATGAG TNAGATTGCA 180

GATTACGAAA CACCATTTCC GCATAGGAAG GGGAATATAT ATNAGATTCA GTACTTGAAT 240

TACTGGAGAG GAGACGTGAA AGAGAAGTAT ATTGAGATNG GTGGAGGAGA GTTTACGGTT 300

GNTATNAGTA AGTTTTTTGG CGAAGTNTNC CNAGAGGNGN CTTNNTNTAA ACCT 354

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 403 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidposis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..403

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

TTTTTTAGTA CACTAATAAT CAAATGGAAT GAGAAATGAA GCCACAAAAG TATCTCCAAT 60

CAAAATATCC TGCTATCTCC ATCTCAAGCT CTCAATAGTA TCCTCTCCGA AAGTGAAATC 120

AACATTTCAA ACTCTATTTC TTGGTGGAAT CGATAGACTG ATTCCTCTGA TGAACCAGAA 180

GTTTCCGGAA CTCGGCTTAC GATCTCAAGA CTGTTCGGAA ATGAGCTGGA TCGAATCGAT 240

AATGTTCTTC AACTGGAGAT CAGGACAGCC GTTAGAGATT TTGCTCAACA GAGACCTAAG 300

GATTCGAGGA TCAGTATTTC AAAGCAAAGT CAGGATTATG GTTCAAAAAC CCGTTCCTGA 360

AAACGTTTTT CGAAGAGGTA TCCAAGGGGT TTCTCGAGCA AGT 403

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..260

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GAGATGAGTT GGATTAANTC TGTACTCTGG TTTGCTGATT TCCCTAAAGG AGAATCTCTT 60

NGTGTTCTCA CGAATCGTAA GCGTACATCT CTATCTTTNA AAGGCAAAGA TGATTTTATC 120

CAAGAACCGA TACCCGAGGC TGCAATTNAA GAGATATGGA GGCGATTAGA AGCCCCCNAG 180

GCTCGGCTTG GAAAGATCAT ATTAACTCCA TTTGGTGGGA AAATNAGTGA AATGGCAGAG 240

TACGTANCAC CATTCCCACA 260

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 605 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..605

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CTCTTGCATA TTCGCTGCAA GGATGGGAAA TTCAAAACCA CTCCCTACAA TTTTTTGTAT 60

TATAGTTTCA GTCTTGTATT TTTAATTCTA TTGCATAACA CCAACTTCTT CATCAGCCTC 120

CATCCAAGAT CAATTCATAA ACTGTGTCAA AAGAAACACA CATGTTTCTT TTCCACTCGA 180

GAAAACGTTA TTCACCCCTG CGAAAAACGT CTCTTTGTTC AACCAAGTCC TTGANTCGAC 240

GGCTCAAAAT CTCCAGTTCT TGGCAAAATC CATGCCTAAA CCGGGRTTCA TATTCAGACC 300

GATTCACCAG TCTCAAGTCC AAGSTTCCAT CATTTGTTCA AMGRAACTCG GGNTTCATTT 360

TNGTGTTTGA NGTGGCGGTC ACGATTTTCG AGGCCTTTGT NTTTATGTTT CACGGTTTGA 420

AAAAACCGTT TATATTACTC GGCCTGTCAA ANTTGNANNC AAAATCANAT GTTGGATATT 480

GNATTCCAAA TAGGTNCTTG GGGTNAACCT GGTGGCTANC GTTTGGTGAG CTTTTACTTT 540

CAAGAATTTG CANGNGGANG TGCAAAGATT CCATGGGATT TCCCGGGGGG TTTNTTGCAC 600

AATGT 605

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 464 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..464

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

AACACAAAAC TCTTCCATTT GGCTTCTCTC TTGCATATTC GTTGCAAGGA TGGGAAATTC 60

AAAACCACTC CCTACAATTN CTTGTATTAT CGTTTCAGTC TTGTATTTTN NATTCTATTG 120

CATAACACCA ACTTCTTCAT CAGCCTCCAT CCAAGNTCAA TTCATAAACT GTGTCAAAAG 180

GAACACACAT GTTTCTTTTC CACTCGAGNA AACGGTATTC ACTCCTGCGG AAAACGGCTC 240

TNTTATTCAA CGGGTCCNTG AATCGACGGG TCAAAATCTC CAGTTCTTGG NAAAATCCAT 300

GNCTAAACCG GGGTTCATAT TCAGGCCGGT TCACCAGTCT CAAGTCCAAG NTTCCATCAT 360

TTGTTCAAAG GAACTCGGGA TTCATTTCCG CGNTAGAAGT GGCGGGCANN GGTTTCGGGG 420

CCTGTCTNTT GNTTANGGGN AGGAAAACCG GTTNTATTNC TCGG 464

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 386 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..386

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

TCGGGAGCCC ANGNTAAATT ANNTGAAAAT GGGGNCGNAT ANCCGTTTAC NGAATTTTAT 60

GACNCCCAAT ATGTTTCGAA ATCTCAAAGA NNGGGANCTT ATGTCAATTT CAAGGATATG 120

GATTTGGGTA TGTATCTTGG AAAGNAGAAG ACAAAGTACG AGGAAGGAAA GAGTTGGGGA 180

GTGAAGTATT TCAAGAACAA TTTCGAGAGA TTGGTGAGAG TGAAGACTAG GGTTGATCCN 240

ACAGATTTCN TCTGCGATGA ACAGAGCATT CCTCTGGTGN ACAAAGTTAC CTGAAGATAT 300

CATTTGAAGT TTTTTATTAG TCCCTTTTCT CTGTGAAATC ATCTGTGCGT GTTGAATANT 360

ATGCGTCAAG TGTGTAACTT ATGTGT 386

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..377

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

TACCATAGGG AGGTGGTGNA AGATTTTGTA TGTAGNCTTA GGGGAAGGCG AGTAGTATGG 60

TGGTGGTGGG GAGCTGTAAA CGTATGGTGG TGGTGGAGAT TTGTATGTGG GCTGGTTAAC 120

TTCATTGAAG CTAAAATCTG GGGACCTAAG TACTTCAAAG GCAATTTTGA CAGATTGGTG 180

AAGATTAAAA CCAAGGTTGA TCCAGAGAAC TTCTTCAGGC ACGAGCAGAG TATCCCACCT 240

ATGCCCTACT AGAAGCTAGG TTCATGAAAC CAATAACATT ATCAAAAATA AGAATAAATG 300

ATAATTGTAT ACAACATGAT TCGTCTTTCT TTATTTCAGA CAATGTGGAC ACTACTCTAA 360

ATAAAATGTC ATTTACC 377

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..377

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

TACCATAGGG AGGTGGTGNA AGATTTTGTA TGTAGNCTTA GGGGAAGGCG AGTAGTATGG 60

TGGTGGTGGG GAGCTGTAAA CGTATGGTGG TGGTGGAGAT TTGTATGTGG GCTGGTTAAC 120

TTCATTGAAG CTAAAATCTG GGGACCTAAG TACTTCAAAG GCAATTTTGA CAGATTGGTG 180

AAGATTAAAA CCAAGGTTGA TCCAGAGAAC TTCTTCAGGC ACGAGCAGAG TATCCCACCT 240

ATGCCCTACT AGAAGCTAGG TTCATGAAAC CAATAACATT ATCAAAAATA AGAATAAATG 300

ATAATTGTAT ACAACATGAT TCGTCTTTCT TTATTTCAGA CAATGTGGAC ACTACTCTAA 360

ATAAAATGTC ATTTACC 377

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 346 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..346

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

GAGCTGTGGA TATGGTCACA AATGGCAATC GGTTGGTCCG AAAACTGATC CGAATCTTTT 60

TATGAGAATN TTGATTCAAC CAGTGACGAG GAAGAAGGTA AAGACTGTGA GAGCTTCTNT 120

GGTTGCCCTN TTTTNAGGCN AGACAGATGA AGTTTTTGCT TTCCTTAGTA AGGAGTTTCC 180

TGAATTGGGT TTAAAGAAGG AGAATTNTTC GGAGATGACT TGGTTTCANT CTGCTTTATG 240

GTGGGACAAT CGTCTTAATG CTACTCAGGT TGATCCTAAA GTNTTTCTTG ATCGGAATCT 300

CGATACCTCG AGTTTCGGTA AGAGGAAATC GGATTACGTC GCGACT 346

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 261 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..261

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

ATGGGGTGAG ACTTATTTCA AAGGTAATTT CAAGAGATTA GGTTTGGTTA AAGGGAAGNT 60

TGATCCAACA AATTTCTTCA GGAACGAACA GAGTATTCCT CCTCTGTTTT GAGTCCTCAA 120

TACAAAACCA GATATAAAAG ATGTCATTTC ATTTTTTCAA TTATAATAGA TAATGTAACT 180

TTCTGCTACA ATTGTAAAAG TGAGATGTAC CCAATACGGT TTAAGCGGAC CGAGAATAGT 240

CAATTCAAAG ACCAAATTCT G 261

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 478 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..478

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

GCTCAAAGGA CTAACCATGA AAACTTCCTC AAGTGTCTCT CTCACCGANT CAACGAGGAC 60

GACTCAAGAN TTATACACAC ATCAAAAGAT CCTTCGTATT TNTCAATCTT GATTTCTTCC 120

ATACAAAATC CAAGTTTCTC TGTTCTCGAA ACACCTAAAC CGGTTTCAAT CATCACTCCG 180

GTTCAAGCCA CCGATGTTCA ATCTACGNTT AAATNCGCAC GGNCTTCACG GGTATACACA 240

ATCAGGGCTA GGAGTGGTNG TCATGACTAC GGAGGTTTAT CTTTACATTG GCTTAAAAAN 300

CANNCCGTTC GTTNNTCATT GATTTNNAGA AATCTTCCGG GCTTATTTAA CATNTAAGAT 360

GTTTGATAAN CCGGNNCCNG TTTGGGGTTC AAATCCCGGT GGCTTACAAA NTTNGGGGGA 420

ATTGTNCCTA TGAGGTTTGG AAAATTAANG CAAAATNTTT TGGGCCTTCC CGGCCGGT 478

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 579 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: ecotype Columbia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..579

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

GGCCGTTAGG ATCATCAAGA AATGGCAATA TGCTGCAGAT AAGGTTCCTG ATGATCTTTT 60

CATTAGGACA ACATTGGAGA GATCAAACAA GAACGCAGTA CACGCTTTGT TCACTGGACT 120

ATATATTGGT CCGGTGAACA ATCTATTGGC GTTGATGGAA GAAAAGTTTC CGGAACTAGG 180

TCTTGAGAAA GAAGGTTGTG AAGAGATGAG TTGGATTGAG TCTGTACTCT GGTTTGCTGA 240

TTTCCCTAAA GGAGAATCTC TTGGTGTTCT CACGAATCGT GAGCGTACAT CTCTATCTTT 300

CAAAGGCAAA GATGATTTTG TCCAAGAACC GATACCCGAG GCTGCAATTC AAGAGATATG 360

GAGGCGATTA GAAGCCCCCG AGGCTCGGCT TGGAAAGATC ATATTAACTC CATTTGGGTG 420

NGGNAAAATG AGTGAAATGG CAGAGNCCGA ACCACCAATT CCCACANNCG AGGGAGGGGA 480

ACCCCTNTGN GGNTCAGAAT GTGGTTCCTG GNNNNNAAGN GGGNGCCAGN ACCAANCCGG 540

GNCNGTAAAN CNTGNAATGG GCCNAACCCG TNCCGGATT 579

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 252 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sativa

(B) STRAIN: Nipponbare, subsp. japonica
(D) DEVELOPMENTAL STAGE: etiolated shoot (8 days old)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..252

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

TGTCCTGGAA GGTCCGCCTC GTGCAGGTTN CGACGACGGT GACGGTGTTC GTCGTCGGGA 60

GGAACGTCGA CCAGGGCGCC GCNGACGTCG TCGCCAGATG GCAAGACGTC GCGCCGAGCC 120

TCCCTCCCGA GCTCACCATA CGGGTGATCG TNCGAGGGCA GCGCGCCACG TTCCAGTCGC 180

TGTACCTCGG CTCGTGCGCC GACCTGGTGC CGACGATGAG CAGCATGTTC CCGGAGCTCG 240

GGATGACGAT TG 252

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactuca sativa
(B) STRAIN: lollo bionda

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /label= Ambiguous
/note= "Xaa = Cys or Ser"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 20..21
(D) OTHER INFORMATION: /label= ambiguous
/note= "Xaa-Xaa probably is Ser-Phe"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Xaa Leu Asn Asn Arg
1 5 10 15

Ala Asp Pro Xaa Xaa
20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactuca sativa
(B) STRAIN: lollo bionda

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 1
(D) OTHER INFORMATION: /label= ambiguous
/note= "Xaa = probably Ser"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 3
(D) OTHER INFORMATION: /label= unknown

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 5
(D) OTHER INFORMATION: /label= ambiguous
/note= "Xaa = probably Ser"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(D) OTHER INFORMATION: /label= ambiguous
/note= "Xaa = probably Trp"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 24
(D) OTHER INFORMATION: /label= ambiguous
/note= "Xaa = probably Tyr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Xaa Ile Xaa Val Xaa Ile Glu Asp Glu Thr Ala Xaa Val Gln Ala Gly
1 5 10 15

Ala Thr Leu Gly Glu Val Tyr Xaa
20

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactuca sativa
(B) STRAIN: lollo bionda

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

Ala Asp Pro Ser Phe Pro Leu Ser Gly Gln Leu Tyr Tyr Pro
1 5 10

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

ACTTCTACTT CTATTATTGA TAGGTTTACT CA 32

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 405 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactuca sativa
(B) STRAIN: lollo bionda

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..405

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

```
ACT TCT ACT TCT ATT ATT GAT AGG TTT ACT CAA TGT CTA AAC AAC CGA     48
Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg
  1               5                  10                  15

GCT GAC CCT TCT TTC CCG CTC AGT GGA CAA CTT TAC ACT CCC GAT AAC     96
Ala Asp Pro Ser Phe Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn
            20                  25                  30

TCC TCT TTT CCA TCC GTC TTG CAA GCT TAC ATC CGG AAC CTC CGA TTC    144
Ser Ser Phe Pro Ser Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe
        35                  40                  45

AAT GAA TCC ACG ACT CCC AAA CCC ATC TTA ATC ATC ACC GCC TTA CAC    192
Asn Glu Ser Thr Thr Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His
    50                  55                  60

CCT TCA CAC ATT CAA GCA GCT GTT GTG TGC GCC AAA ACA CAC CGC CTG    240
Pro Ser His Ile Gln Ala Ala Val Val Cys Ala Lys Thr His Arg Leu
65                  70                  75                  80

CTA ATG AAA ACC AGA AGC GGA GGC CAT GAT TAT GAG GGG CTT TCC TAT    288
Leu Met Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr
                85                  90                  95

GTG ACC AAT TCG AAC CAA CCC TTT TTT GTT GTT GAC ATG TTC AAC TTA    336
Val Thr Asn Ser Asn Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu
            100                 105                 110

CGC TCC ATA AAC GTG AGT ATT GAA GAT GAA ACT GCA TGG GTC CAA GCC    384
Arg Ser Ile Asn Val Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala
        115                 120                 125

GGC GCC ACC CTC GGA GAA GTT                                       405
Gly Ala Thr Leu Gly Glu Val
    130                 135
```

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 135 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

```
Thr Ser Thr Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg
  1               5                  10                  15
```

Ala Asp Pro Ser Phe Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn
20 25 30

Ser Ser Phe Pro Ser Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe
35 40 45

Asn Glu Ser Thr Thr Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His
50 55 60

Pro Ser His Ile Gln Ala Ala Val Val Cys Ala Lys Thr His Arg Leu
65 70 75 80

Leu Met Lys Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr
85 90 95

Val Thr Asn Ser Asn Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu
100 105 110

Arg Ser Ile Asn Val Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala
115 120 125

Gly Ala Thr Leu Gly Glu Val
130 135

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

CACGTTTATG GAGCGTAAGT TGAAC 25

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

CACCCTTCAC ACATTCAAGC AGC 23

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1981 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lactuca sativa
(B) STRAIN: lollo bionda

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 7..1626

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: replace(372, "g")

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: replace(379, "g")

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: replace(786, "t")

(ix) FEATURE:
(A) NAME/KEY: unsure
(B) LOCATION: replace(1105..1106, "ga")
(D) OTHER INFORMATION: /note= "also possible "gg" and "aa""

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

```
ACAAAA ATG GCA ATT ACC TAT TCT TTC AAC TTC AAA TCT TAT ATT TTT      48
       Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe
         1               5                  10

CCT CTC CTC CTT GTC TTG CTC TCT ACC CAT TCA TCA GCG ACT TCA ACT     96
Pro Leu Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr
 15                  20                  25                  30

TCC ATT ATA GAT CGC TTC ACC CAA TGT CTA AAC AAC CGA GCT GAC CCT    144
Ser Ile Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro
                 35                  40                  45
```

```
TCT TTC CCG CTC AGT GGA CAA CTT TAC ACT CCC GAT AAC TCC TCT TTT   192
Ser Phe Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn Ser Ser Phe
                50                  55                  60

CCA TCC GTC TTG CAA GCT TAC ATC CGG AAC CTC CGA TTC AAT GAA TCC   240
Pro Ser Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe Asn Glu Ser
            65                  70                  75

ACG ACT CCC AAA CCC ATC TTA ATC ATC ACC GCC TTA CAC CCT TCA CAC   288
Thr Thr Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His Pro Ser His
        80                  85                  90

ATT CAA GCA GCT GTT GTG TGC GCC AAA ACA CAC CGC CTG CTA ATG AAA   336
Ile Gln Ala Ala Val Val Cys Ala Lys Thr His Arg Leu Leu Met Lys
    95                  100                 105                 110

ACC AGA AGC GGA GGC CAT GAT TAT GAG GGG CTT TCC TAT GTG ACC AAT   384
Thr Arg Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Val Thr Asn
                115                 120                 125

TCG AAC CAA CCC TTT TTT GTT GTT GAC ATG TTC AAC TTA CGC TCC ATA   432
Ser Asn Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu Arg Ser Ile
            130                 135                 140

AAC GTG AGT ATT GAA GAT GAA ACT GCA TGG GTC CAA GCT GGT GCG ACT   480
Asn Val Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala Gly Ala Thr
        145                 150                 155

CTT GGT GAA GTC TAC TAC CGA ATA GCA GAG AAA AGC AAC AGT CAT GCT   528
Leu Gly Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Ser His Ala
    160                 165                 170

TTT CCG GCT GGC GTT TGC CCT ACT GTT GGA GTT GGT GGC CAT TTT AGT   576
Phe Pro Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser
175                 180                 185                 190

GGT GGT GGT TAT GGT AAC TTG ATG GGA AAA TAC GGC CTT TCT GTT GAC   624
Gly Gly Gly Tyr Gly Asn Leu Met Gly Lys Tyr Gly Leu Ser Val Asp
                195                 200                 205

AAT ATT GTC GAT GCT CAG TTA ATC GAT GTG AAT GGT AAA CTT CTG AAT   672
Asn Ile Val Asp Ala Gln Leu Ile Asp Val Asn Gly Lys Leu Leu Asn
            210                 215                 220

CGG AAA TCA ATG GGT GAA GAT CTT TTT TGG GCC ATC ACA GGT GGT GGT   720
Arg Lys Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly
        225                 230                 235

GGT GTC AGC TTT GGT GTG GTT GTA GCG TAC AAG ATC AAA CTG GTT CGT   768
Gly Val Ser Phe Gly Val Val Val Ala Tyr Lys Ile Lys Leu Val Arg
    240                 245                 250

GTT CCT ACC ACT GTG ACC GTT TTT AAC GTA CAA AGA ACA TCC GAG CAG   816
Val Pro Thr Thr Val Thr Val Phe Asn Val Gln Arg Thr Ser Glu Gln
255                 260                 265                 270
```

```
AAC CTA AGC ACC ATA GCC CAC CGA TGG ATA CAA GTT GCG GAT AAG CTC   864
Asn Leu Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu
                275                 280                 285

GAT AAT GAC CTT TTC CTT CGA ATG ACC TTT AAC GTG ATA AAC AAC ACA   912
Asp Asn Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr
            290                 295                 300

AAT GGC GAA AAG ACG ATA CGT GGT TTG TTT CCA ACA CTG TAC CTC GGA   960
Asn Gly Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly
        305                 310                 315

AAC TCT ACC GCT CTT GTT GCC CTC CTG AAC AAG GAT TTC CCT GAA TTA  1008
Asn Ser Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu
    320                 325                 330

GGT GTA GAA ATT TCA GAT TGT ATT GAA ATG AGT TGG ATC GAG TCT GTT  1056
Gly Val Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val
335                 340                 345                 350

CTT TTC TAC ACA AAC TTC CCC ATT GGT ACT CCG ACC ACT GCT CTT CTA  1104
Leu Phe Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu
                355                 360                 365

AGC CGT ACA CCT CAA AGA CTA AAC CCA TTC AAA ATC AAA TCT GAT TAC  1152
Ser Arg Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr
            370                 375                 380

GTA AAA AAC ACT ATT TCC AAA CAG GGA TTC GAA TCC ATA TTT GAA AGG  1200
Val Lys Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg
        385                 390                 395

ATG AAA GAA CTC GAA AAC CAA ATG CTA GCT TTC AAC CCT TAT GGT GGA  1248
Met Lys Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly
    400                 405                 410

AGA ATG AGC GAA ATT TCC GAA TTT GCA AAG CCT TTT CCC CAT CGA TCA  1296
Arg Met Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser
415                 420                 425                 430

GGG AAT ATA GCG AAG ATC CAA TAC GAA GTA AAC TGG GAT GAA CTT GGC  1344
Gly Asn Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly
                435                 440                 445

GTT GAA GCA GCC AAT CGG TAC TTG AAC TTC ACA AGG GTG ATG TAT GAT  1392
Val Glu Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp
            450                 455                 460

TAT ATG ACT CCG TTT GTT TCT AAG AAC CCC AGG GAA GCA TTT CTG AAC  1440
Tyr Met Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn
        465                 470                 475

TAC AGG GAT TTA GAT ATT GGT GTC AAC AGT CAT GGC AAG AAT GCT TAC  1488
Tyr Arg Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr
    480                 485                 490
```

GGT GAA GGA ATG GTT TAT GGG CAC AAG TAT TTC AAA GAG ACG AAT TAT 1536
Gly Glu Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr
495 500 505 510

AAG AGG CTA ACG ATG GTG AAG ACG AGG GTT GAT CCT AGC AAT TTT TTT 1584
Lys Arg Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe
515 520 525

AGG AAT GAG CAA AGT ATC CCA ACT TTG TCA TCT TCA TGG AAG 1626
Arg Asn Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys
530 535 540

TAAATTCTAA ATTCACTTGT GAAATTGAAT AAAAGTATGG CTTTTTCAAG GTCATGGTAT 1686

CCAGATTCAG ATGATATTGA TATAATTTTG ACTTGTATTT ATACAAACAA AATTATATTA 1746

TATTTTTCTG AATTTAGATT TTCCATTCTT TGGAAAAATA TACGAACATT GATGTTGATA 1806

TTTTTAAGAA TTATAGATTT TGAACATTGT GAACAATGAA TAAACCGAGG ACTTCCCTTG 1866

GGTTTTTTTT ATAAGTATGT AATAGCATGT CTTTAATCAA GATAACCGAT CATTGGATGC 1926

AATTTATTAT TATAAACCTT ATTTAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAA 1981

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 540 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Met Ala Ile Thr Tyr Ser Phe Asn Phe Lys Ser Tyr Ile Phe Pro Leu
1 5 10 15

Leu Leu Val Leu Leu Ser Thr His Ser Ser Ala Thr Ser Thr Ser Ile
20 25 30

Ile Asp Arg Phe Thr Gln Cys Leu Asn Asn Arg Ala Asp Pro Ser Phe
35 40 45

Pro Leu Ser Gly Gln Leu Tyr Thr Pro Asp Asn Ser Ser Phe Pro Ser
50 55 60

Val Leu Gln Ala Tyr Ile Arg Asn Leu Arg Phe Asn Glu Ser Thr Thr
65 70 75 80

Pro Lys Pro Ile Leu Ile Ile Thr Ala Leu His Pro Ser His Ile Gln
85 90 95

Ala Ala Val Val Cys Ala Lys Thr His Arg Leu Leu Met Lys Thr Arg
100 105 110

Ser Gly Gly His Asp Tyr Glu Gly Leu Ser Tyr Val Thr Asn Ser Asn
115 120 125

Gln Pro Phe Phe Val Val Asp Met Phe Asn Leu Arg Ser Ile Asn Val
130 135 140

Ser Ile Glu Asp Glu Thr Ala Trp Val Gln Ala Gly Ala Thr Leu Gly
145 150 155 160

Glu Val Tyr Tyr Arg Ile Ala Glu Lys Ser Asn Ser His Ala Phe Pro
165 170 175

Ala Gly Val Cys Pro Thr Val Gly Val Gly Gly His Phe Ser Gly Gly
180 185 190

Gly Tyr Gly Asn Leu Met Gly Lys Tyr Gly Leu Ser Val Asp Asn Ile
195 200 205

Val Asp Ala Gln Leu Ile Asp Val Asn Gly Lys Leu Leu Asn Arg Lys
210 215 220

Ser Met Gly Glu Asp Leu Phe Trp Ala Ile Thr Gly Gly Gly Gly Val
225 230 235 240

Ser Phe Gly Val Val Val Ala Tyr Lys Ile Lys Leu Val Arg Val Pro
245 250 255

Thr Thr Val Thr Val Phe Asn Val Gln Arg Thr Ser Glu Gln Asn Leu
260 265 270

Ser Thr Ile Ala His Arg Trp Ile Gln Val Ala Asp Lys Leu Asp Asn
275 280 285

Asp Leu Phe Leu Arg Met Thr Phe Asn Val Ile Asn Asn Thr Asn Gly
290 295 300

Glu Lys Thr Ile Arg Gly Leu Phe Pro Thr Leu Tyr Leu Gly Asn Ser
305 310 315 320

Thr Ala Leu Val Ala Leu Leu Asn Lys Asp Phe Pro Glu Leu Gly Val
325 330 335

Glu Ile Ser Asp Cys Ile Glu Met Ser Trp Ile Glu Ser Val Leu Phe
340 345 350

Tyr Thr Asn Phe Pro Ile Gly Thr Pro Thr Thr Ala Leu Leu Ser Arg
355 360 365

Thr Pro Gln Arg Leu Asn Pro Phe Lys Ile Lys Ser Asp Tyr Val Lys
370 375 380

Asn Thr Ile Ser Lys Gln Gly Phe Glu Ser Ile Phe Glu Arg Met Lys
385 390 395 400

Glu Leu Glu Asn Gln Met Leu Ala Phe Asn Pro Tyr Gly Gly Arg Met
405 410 415

Ser Glu Ile Ser Glu Phe Ala Lys Pro Phe Pro His Arg Ser Gly Asn
420 425 430

Ile Ala Lys Ile Gln Tyr Glu Val Asn Trp Asp Glu Leu Gly Val Glu
435 440 445

Ala Ala Asn Arg Tyr Leu Asn Phe Thr Arg Val Met Tyr Asp Tyr Met
450 455 460

Thr Pro Phe Val Ser Lys Asn Pro Arg Glu Ala Phe Leu Asn Tyr Arg
465 470 475 480

Asp Leu Asp Ile Gly Val Asn Ser His Gly Lys Asn Ala Tyr Gly Glu
485 490 495

Gly Met Val Tyr Gly His Lys Tyr Phe Lys Glu Thr Asn Tyr Lys Arg
500 505 510

Leu Thr Met Val Lys Thr Arg Val Asp Pro Ser Asn Phe Phe Arg Asn
515 520 525

Glu Gln Ser Ile Pro Thr Leu Ser Ser Ser Trp Lys
530 535 540

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GGTAATGATC TCCTTTCTTG TTTGACC 27

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

AGAGCGGCCG CTATATTACA ACTTCTCCAC CATCACTCCT C 41

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GGTGATGTTA ATGATAATCT CCTC 24

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

AGAGCGGCCG CTACAATTCC TTCAACATGT AAATTTCCTC 40

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

ACTTCCCGTA GAAACTCGGA GACTTTCACA CAATGC 36

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

TCCATCCAAG ATCAATTCAT AAACTGTGTC 30

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

AGAGCGGCCG CTTTCATGAA CCTAGCTTCT AGTAGG 36

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

AGAGCGGCCG CGAAATGGCC CCCCTTTTAA AACGGGG 37

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

AGAGCGGCCG CAAATGATAT CTTCAGGTAA CTTTGTTCAC 40

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

AGAGCGGCCG CATAATCAAA TAAATACACT TATGGTAACA CAG 43

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

AGAGCGGCCG CTGGTTTTGT ATTGAGGACT CAAAACAG 38

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1757 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: Colombia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: join(1..570, 801..1754)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

```
ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC CTA ACC TCA AAC     48
Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn
  1               5                  10                  15

TCC GAC CCC AAA CAT CCC ATC TCC CCC GCT ATC TTC TTC TCC GGA AAT     96
Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn
            20                  25                  30

GGC TCC TAC TCC TCC GTA TTA CAA GCC AAC ATC CGT AAC CTC CGC TTC    144
Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe
        35                  40                  45

AAC ACC ACC TCA ACT CCG AAA CCC TTC CTC ATA ATC GCC GCA ACA CAT    192
Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His
    50                  55                  60

GAA TCC CAT GTG CAA GCC GCG ATT ACT TGC GGG AAA CGC CAC AAC CTT    240
Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu
65                  70                  75                  80

CAG ATG AAA ATC AGA AGT GGA GGC CAC GAC TAC GAT GGC TTG TCA TAC    288
Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr
                85                  90                  95

GTT ACA TAC TCT GGC AAA CCG TTC TTC GTC CTC GAC ATG TTT AAC CTC    336
Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu
            100                 105                 110

CGT TCG GTG GAT GTC GAT GTG GCA AGT AAG ACC GCG TGG GTC CAA ACC    384
Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr
        115                 120                 125

GGT GCC ATA CTC GGA GAA GTT TAT TAC TAT ATA TGG GAG AAG AGC AAA    432
Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys
    130                 135                 140

ACC CTA GCT TAT CCC GCC GGA ATT TGT CCC ACG GTT GGT GTC GGT GGC    480
Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly
145                 150                 155                 160

CAT ATC AGT GGT GGA GGT TAC GGT AAC ATG ATG AGA AAA TAC GGT CTC    528
His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu
                165                 170                 175
```

```
ACC GTA GAT AAT ACC ATC GAT GCA AGA ATG GTC GAC GTT AAT                570
Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn
            180                 185                 190

GGTATAATTG ATATCTCTAT TTTATATACT AATTAAATTT TATAGTGTGG ATCGGATAGT  630

GATTTTGGTC CATCAATTAA AAACTTGGTG AACATAAAAT TAACCAAGCA ATCAATTTAG  690

ACAAGCAACA TAATCATATA TATTTTTCTT ACATTTGTAT GTACCTGAAT ATTTATATTT  750

ATGTTTATAT GTTCTCACTA TATTTTCACT TTTGTATTTG AAAATTTTTA GGA AAA      806
                                                        Gly Lys

ATT TTG GAT AGA AAA TTG ATG GGA GAA GAT CTC TAC TGG GCA ATA AAC     854
Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn
        195                 200                 205

GGA GGA GGA GGA GGG AGC TAC GGC GTC GTA TTG GCC TAC AAA ATA AAC     902
Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn
    210                 215                 220

CTT GTT GAA GTC CCA GAA AAC GTC ACC GTT TTC AGA ATC TCC CGG ACG     950
Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr
225                 230                 235                 240

TTA GAA CAA AAT GCG ACG GAT ATC ATT CAC CGG TGG CAA CAA GTT GCA     998
Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala
                245                 250                 255

CCG AAG CTT CCC GAC GAG CTT TTC ATA AGA ACA GTC ATT GAC GTA GTA    1046
Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val
            260                 265                 270

AAC GGC ACT GTT TCA TCT CAA AAG ACC GTC AGG ACA ACA TTC ATA GCA    1094
Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala
        275                 280                 285

ATG TTT CTA GGA GAC ACG ACA ACT CTA CTG TCG ATA TTA AAC CGG AGA    1142
Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg
    290                 295                 300

TTC CCA GAA TTG GGT TTG GTC CGG TCT GAC TGT ACC GAA ACA AGC TGG    1190
Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp
305                 310                 315                 320

ATC CAA TCT GTG CTA TTC TGG ACA AAT ATC CAA GTT GGT TCG TCG GAG    1238
Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu
                325                 330                 335

ACA CTT CTA CTC CAA AGG AAT CAA CCC GTG AAC TAC CTC AAG AGG AAA    1286
Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys
            340                 345                 350
```

```
TCA GAT TAC GTA CGT GAA CCG ATT TCA AGA ACC GGT TTA GAG TCA ATT  1334
Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile
        355                 360                 365

TGG AAG AAA ATG ATC GAG CTT GAA ATT CCG ACA ATG GCT TTC AAT CCA  1382
Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro
    370                 375                 380

TAC GGT GGT GAG ATG GGG AGG ATA TCA TTA CGG GTG ACT CCG TTC CCA  1430
Tyr Gly Gly Glu Met Gly Arg Ile Ser Leu Arg Val Thr Pro Phe Pro
385                 390                 395                 400

TAC AGA GCC GGT AAT CTC TGG AAG ATT CAG TAC GGT GCG AAT TGG AGA  1478
Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg
                405                 410                 415

GAT GAG ACT TTA ACC GAC CGG TAC ATG GAA TTG ACG AGG AAG TTG TAC  1526
Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr
            420                 425                 430

CAA TTC ATG ACA CCA TTT GTT TCC AAG AAT CCG AGA CAA TCG TTT TTC  1574
Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe
        435                 440                 445

AAT AAC CGT GAT GTT GAT TTG GGT ATT AAT TCT CAT AAT GGT AAA ATC  1622
Asn Asn Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile
    450                 455                 460

AGT AGT TAT GTG GAA GGT AAA CGT TAC GGG AAG AAG TAT TTC GCA GGT  1670
Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly
465                 470                 475                 480

AAT TTC GAG AGA TTG GTG AAG ATT AAG ACG AGA GTT GAT AGT GGT AAT  1718
Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn
                485                 490                 495

TTC TTT AGG AAC GAA CAC AGT ATT CCT GTG TTA CCA TAA              1757
Phe Phe Arg Asn Glu His Ser Ile Pro Val Leu Pro
            500                 505
```

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

```
Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn
 1               5                  10                  15
```

```
Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn
                20                  25                  30

Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe
            35                  40                  45

Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His
        50                  55                  60

Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu
65                  70                  75                  80

Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr
                85                  90                  95

Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu
            100                 105                 110

Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr
        115                 120                 125

Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys
    130                 135                 140

Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly
145                 150                 155                 160

His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu
                165                 170                 175

Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn Gly Lys
            180                 185                 190

Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn
        195                 200                 205

Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn
    210                 215                 220

Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr
225                 230                 235                 240

Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala
                245                 250                 255

Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val
            260                 265                 270

Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala
        275                 280                 285

Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg
    290                 295                 300
```

```
Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp
305                 310                 315                 320

Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu
                325                 330                 335

Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys
            340                 345                 350

Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile
        355                 360                 365

Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro
    370                 375                 380

Tyr Gly Gly Glu Met Gly Arg Ile Ser Leu Arg Val Thr Pro Phe Pro
385                 390                 395                 400

Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg
                405                 410                 415

Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr
            420                 425                 430

Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe
        435                 440                 445

Asn Asn Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile
    450                 455                 460

Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly
465                 470                 475                 480

Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn
                485                 490                 495

Phe Phe Arg Asn Glu His Ser Ile Pro Val Leu Pro
            500                 505
```

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1527 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: Colombia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1524

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

```
ACT TCC CGT AGA AAC TCG GAG ACT TTC ACA CAA TGC CTA ACC TCA AAC      48
Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn
  1               5                  10                  15

TCC GAC CCC AAA CAT CCC ATC TCC CCC GCT ATC TTC TTC TCC GGA AAT      96
Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn
            20                  25                  30

GGC TCC TAC TCC TCC GTA TTA CAA GCC AAC ATC CGT AAC CTC CGC TTC     144
Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe
        35                  40                  45

AAC ACC ACC TCA ACT CCG AAA CCC TTC CTC ATA ATC GCC GCA ACA CAT     192
Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His
    50                  55                  60

GAA TCC CAT GTG CAA GCC GCG ATT ACT TGC GGG AAA CGC CAC AAC CTT     240
Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu
65                  70                  75                  80

CAG ATG AAA ATC AGA AGT GGA GGC CAC GAC TAC GAT GGC TTG TCA TAC     288
Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr
                85                  90                  95

GTT ACA TAC TCT GGC AAA CCG TTC TTC GTC CTC GAC ATG TTT AAC CTC     336
Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu
            100                 105                 110

CGT TCG GTG GAT GTC GAC GTG GCA AGT AAG ACC GCG TGG GTC CAA ACC     384
Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr
        115                 120                 125

GGT GCC ATA CTC GGA GAA GTT TAT TAC TAT ATA TGG GAG AAG AGC AAA     432
Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys
    130                 135                 140

ACC CTA GCT TAT CCC GCC GGA ATT TGT CCC ACG GTT GGT GTC GGT GGC     480
Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly
145                 150                 155                 160

CAT ATC AGT GGT GGA GGT TAC GGT AAC ATG ATG AGA AAA TAC GGT CTC     528
His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu
                165                 170                 175
```

```
ACC GTA GAT AAT ACC ATC GAT GCA AGA ATG GTC GAC GTA AAT GGA AAA   576
Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn Gly Lys
            180                 185                 190

ATT TTG GAT AGA AAA TTG ATG GGA GAA GAT CTC TAC TGG GCA ATA AAC   624
Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn
        195                 200                 205

GGA GGA GGA GGA GGG AGC TAC GGC GTC GTA TTG GCC TAC AAA ATA AAC   672
Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn
    210                 215                 220

CTT GTT GAA GTC CCA GAA AAC GTC ACC GTT TTC AGA ATC TCC CGG ACG   720
Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr
225                 230                 235                 240

TTA GAA CAA AAT GCG ACG GAT ATC ATT CAC CGG TGG CAA CAA GTT GCA   768
Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala
                245                 250                 255

CCG AAG CTT CCC GAC GAG CTT TTC ATA AGA ACA GTC ATT GAC GTA GTA   816
Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val
            260                 265                 270

AAC GGC ACT GTT TCA TCT CAA AAG ACC GTC AGG ACA ACA TTC ATA GCA   864
Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala
        275                 280                 285

ATG TTT CTA GGA GAC ACG ACA ACT CTA CTG TCG ATA TTA AAC CGG AGA   912
Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg
    290                 295                 300

TTC CCA GAA TTG GGT TTG GTC CGG TCT GAC TGT ACC GAA ACA AGC TGG   960
Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp
305                 310                 315                 320

ATC CAA TCT GTG CTA TTC TGG ACA AAT ATC CAA GTT GGT TCG TCG GAG  1008
Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu
                325                 330                 335

ACA CTT CTA CTC CAA AGG AAT CAA CCC GTG AAC TAC CTC AAG AGG AAA  1056
Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys
            340                 345                 350

TCA GAT TAC GTA CGT GAA CCG ATT TCA AGA ACC GGT TTA GAG TCA ATT  1104
Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile
        355                 360                 365

TGG AAG AAA ATG ATC GAG CTT GAA ATT CCG ACA ATG GCT TTC AAT CCA  1152
Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro
    370                 375                 380

TAC GGT GGT GAG ATG GGG AGG ATA TCA TCT ACG GTG ACT CCG TTC CCA  1200
Tyr Gly Gly Glu Met Gly Arg Ile Ser Ser Thr Val Thr Pro Phe Pro
385                 390                 395                 400
```

```
TAC AGA GCC GGT AAT CTC TGG AAG ATT CAG TAC GGT GCG AAT TGG AGA  1248
Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg
                405                 410                 415

GAT GAG ACT TTA ACC GAC CGG TAC ATG GAA TTG ACG AGG AAG TTG TAC  1296
Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr
            420                 425                 430

CAA TTC ATG ACA CCA TTT GTT TCC AAG AAT CCG AGA CAA TCG TTT TTC  1344
Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe
        435                 440                 445

AAT TAC CGT GAT GTT GAT TTG GGT ATT AAT TCT CAT AAT GGT AAA ATC  1392
Asn Tyr Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile
    450                 455                 460

AGT AGT TAT GTG GAA GGT AAA CGT TAC GGG AAG AAG TAT TTC GCA GGT  1440
Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly
465                 470                 475                 480

AAT TTC GAG AGA TTG GTG AAG ATT AAG ACG AGA GTT GAT AGT GGT AAT  1488
Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn
                485                 490                 495

TTC TTT AGG AAC GAA CAG AGT ATT CCT GTG TTA CCA TAA              1527
Phe Phe Arg Asn Glu Gln Ser Ile Pro Val Leu Pro
            500                 505
```

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

```
Thr Ser Arg Arg Asn Ser Glu Thr Phe Thr Gln Cys Leu Thr Ser Asn
  1               5                  10                  15

Ser Asp Pro Lys His Pro Ile Ser Pro Ala Ile Phe Phe Ser Gly Asn
            20                  25                  30

Gly Ser Tyr Ser Ser Val Leu Gln Ala Asn Ile Arg Asn Leu Arg Phe
        35                  40                  45

Asn Thr Thr Ser Thr Pro Lys Pro Phe Leu Ile Ile Ala Ala Thr His
    50                  55                  60

Glu Ser His Val Gln Ala Ala Ile Thr Cys Gly Lys Arg His Asn Leu
65                  70                  75                  80
```

```
Gln Met Lys Ile Arg Ser Gly Gly His Asp Tyr Asp Gly Leu Ser Tyr
                85                  90                  95

Val Thr Tyr Ser Gly Lys Pro Phe Phe Val Leu Asp Met Phe Asn Leu
            100                 105                 110

Arg Ser Val Asp Val Asp Val Ala Ser Lys Thr Ala Trp Val Gln Thr
        115                 120                 125

Gly Ala Ile Leu Gly Glu Val Tyr Tyr Tyr Ile Trp Glu Lys Ser Lys
    130                 135                 140

Thr Leu Ala Tyr Pro Ala Gly Ile Cys Pro Thr Val Gly Val Gly Gly
145                 150                 155                 160

His Ile Ser Gly Gly Gly Tyr Gly Asn Met Met Arg Lys Tyr Gly Leu
                165                 170                 175

Thr Val Asp Asn Thr Ile Asp Ala Arg Met Val Asp Val Asn Gly Lys
            180                 185                 190

Ile Leu Asp Arg Lys Leu Met Gly Glu Asp Leu Tyr Trp Ala Ile Asn
        195                 200                 205

Gly Gly Gly Gly Gly Ser Tyr Gly Val Val Leu Ala Tyr Lys Ile Asn
    210                 215                 220

Leu Val Glu Val Pro Glu Asn Val Thr Val Phe Arg Ile Ser Arg Thr
225                 230                 235                 240

Leu Glu Gln Asn Ala Thr Asp Ile Ile His Arg Trp Gln Gln Val Ala
                245                 250                 255

Pro Lys Leu Pro Asp Glu Leu Phe Ile Arg Thr Val Ile Asp Val Val
            260                 265                 270

Asn Gly Thr Val Ser Ser Gln Lys Thr Val Arg Thr Thr Phe Ile Ala
        275                 280                 285

Met Phe Leu Gly Asp Thr Thr Thr Leu Leu Ser Ile Leu Asn Arg Arg
    290                 295                 300

Phe Pro Glu Leu Gly Leu Val Arg Ser Asp Cys Thr Glu Thr Ser Trp
305                 310                 315                 320

Ile Gln Ser Val Leu Phe Trp Thr Asn Ile Gln Val Gly Ser Ser Glu
                325                 330                 335

Thr Leu Leu Leu Gln Arg Asn Gln Pro Val Asn Tyr Leu Lys Arg Lys
            340                 345                 350

Ser Asp Tyr Val Arg Glu Pro Ile Ser Arg Thr Gly Leu Glu Ser Ile
        355                 360                 365
```

```
Trp Lys Lys Met Ile Glu Leu Glu Ile Pro Thr Met Ala Phe Asn Pro
    370                 375                 380

Tyr Gly Gly Glu Met Gly Arg Ile Ser Ser Thr Val Thr Pro Phe Pro
385                 390                 395                 400

Tyr Arg Ala Gly Asn Leu Trp Lys Ile Gln Tyr Gly Ala Asn Trp Arg
                405                 410                 415

Asp Glu Thr Leu Thr Asp Arg Tyr Met Glu Leu Thr Arg Lys Leu Tyr
            420                 425                 430

Gln Phe Met Thr Pro Phe Val Ser Lys Asn Pro Arg Gln Ser Phe Phe
        435                 440                 445

Asn Tyr Arg Asp Val Asp Leu Gly Ile Asn Ser His Asn Gly Lys Ile
    450                 455                 460

Ser Ser Tyr Val Glu Gly Lys Arg Tyr Gly Lys Lys Tyr Phe Ala Gly
465                 470                 475                 480

Asn Phe Glu Arg Leu Val Lys Ile Lys Thr Arg Val Asp Ser Gly Asn
                485                 490                 495

Phe Phe Arg Asn Glu Gln Ser Ile Pro Val Leu Pro
            500                 505
```

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1530 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana
(B) STRAIN: Colombia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

```
TCC ATC CAA GAT CAA TTC ATA AAC TGT GTC AAA AGA AAC ACA CAT GTT    48
Ser Ile Gln Asp Gln Phe Ile Asn Cys Val Lys Arg Asn Thr His Val
 1               5                  10                  15
```

```
TCT TTT CCA CTC GAG AAA ACG TTA TTC ACC CCT GCG AAA AAC GTC TCT    96
Ser Phe Pro Leu Glu Lys Thr Leu Phe Thr Pro Ala Lys Asn Val Ser
            20                  25                  30

TTG TTC AAC CAA GTC CTT GAA TCG ACG GCT CAA AAT CTC CAG TTC TTG   144
Leu Phe Asn Gln Val Leu Glu Ser Thr Ala Gln Asn Leu Gln Phe Leu
        35                  40                  45

GCA AAA TCC ATG CCT AAA CCG GGA TTC ATA TTC AGA CCG ATT CAC CAG   192
Ala Lys Ser Met Pro Lys Pro Gly Phe Ile Phe Arg Pro Ile His Gln
    50                  55                  60

TCT CAA GTC CAA GCT TCC ATC ATT TGT TCA AAG AAA CTC GGA ATT CAT   240
Ser Gln Val Gln Ala Ser Ile Ile Cys Ser Lys Lys Leu Gly Ile His
65                  70                  75                  80

TTT CGT GTT AGA AGT GGC GGT CAC GAT TTC GAG GCC TTG TCT TAT GTT   288
Phe Arg Val Arg Ser Gly Gly His Asp Phe Glu Ala Leu Ser Tyr Val
                85                  90                  95

TCA CGG ATT GAA AAA CCG TTT ATA TTA CTC GAC CTG TCA AAA TTG AAA   336
Ser Arg Ile Glu Lys Pro Phe Ile Leu Leu Asp Leu Ser Lys Leu Lys
            100                 105                 110

CAA ATC AAT GTT GAT ATT GAA TCC AAT AGT GCT TGG GTT CAA CCT GGT   384
Gln Ile Asn Val Asp Ile Glu Ser Asn Ser Ala Trp Val Gln Pro Gly
        115                 120                 125

GCT ACG CTT GGT GAG CTT TAC TAC AGA ATT GCA GAG AAG AGC AAG ATC   432
Ala Thr Leu Gly Glu Leu Tyr Tyr Arg Ile Ala Glu Lys Ser Lys Ile
    130                 135                 140

CAT GGA TTT CCC GCG GGT TTG TGC ACA AGT GTA GGC ATA GGT GGG TAT   480
His Gly Phe Pro Ala Gly Leu Cys Thr Ser Val Gly Ile Gly Gly Tyr
145                 150                 155                 160

ATG ACA GGC GGT GGA TAC GGT ACC TTG ATG AGG AAG TAT GGT CTT GCG   528
Met Thr Gly Gly Gly Tyr Gly Thr Leu Met Arg Lys Tyr Gly Leu Ala
                165                 170                 175

GGA GAT AAT GTT CTA GAC GTA AAG ATG GTT GAT GCA AAT GGT AAA TTA   576
Gly Asp Asn Val Leu Asp Val Lys Met Val Asp Ala Asn Gly Lys Leu
            180                 185                 190

CTC GAC AGA GCC GCG ATG GGT GAG GAC CTA TTT TGG GCG ATT AGA GGA   624
Leu Asp Arg Ala Ala Met Gly Glu Asp Leu Phe Trp Ala Ile Arg Gly
        195                 200                 205

GGC GGT GGA GCG AGT TTC GGG ATA GTT CTA GCA TGG AAG ATC AAG CTT   672
Gly Gly Gly Ala Ser Phe Gly Ile Val Leu Ala Trp Lys Ile Lys Leu
    210                 215                 220

GTT CCT GTT CCT AAG ACT GTT ACC GTC TTC ACT GTC ACC AAA ACG TTA   720
Val Pro Val Pro Lys Thr Val Thr Val Phe Thr Val Thr Lys Thr Leu
225                 230                 235                 240
```

```
GAA CAA GAC GCA AGA TTG AAG ACT ATT TCT AAG TGG CAA CAA ATT TCA   768
Glu Gln Asp Ala Arg Leu Lys Thr Ile Ser Lys Trp Gln Gln Ile Ser
                245                 250                 255

TCC AAG ATT ATT GAA GAG ATA CAC ATC CGA GTG GTA CTC AGA GCA GCT   816
Ser Lys Ile Ile Glu Glu Ile His Ile Arg Val Val Leu Arg Ala Ala
            260                 265                 270

GGA AAT GAT GGA AAC AAG ACT GTG ACA ATG ACC TAC CTA GGT CAG TTT   864
Gly Asn Asp Gly Asn Lys Thr Val Thr Met Thr Tyr Leu Gly Gln Phe
        275                 280                 285

CTT GGC GAG AAA GGC ACC TTG CTG AAG GTT ATG GAG AAG GCT TTT CCA   912
Leu Gly Glu Lys Gly Thr Leu Leu Lys Val Met Glu Lys Ala Phe Pro
    290                 295                 300

GAA CTA GGG TTA ACT CAA AAG GAT TGT ACT GAA ATG AGC TGG ATT GAA   960
Glu Leu Gly Leu Thr Gln Lys Asp Cys Thr Glu Met Ser Trp Ile Glu
305                 310                 315                 320

GCC GCC CTT TTC CAT GGT GGA TTT CCA ACA GGT TCT CCT ATT GAA ATT  1008
Ala Ala Leu Phe His Gly Gly Phe Pro Thr Gly Ser Pro Ile Glu Ile
                325                 330                 335

TTG CTT CAG CTC AAG TCG CCT CTA GGA AAA GAT TAC TTC AAA GCA ACG  1056
Leu Leu Gln Leu Lys Ser Pro Leu Gly Lys Asp Tyr Phe Lys Ala Thr
            340                 345                 350

TCG GAT TTC GTT AAA GAA CCT ATT CCT GTG ATA GGC TTC AAA GGA ATA  1104
Ser Asp Phe Val Lys Glu Pro Ile Pro Val Ile Gly Phe Lys Gly Ile
        355                 360                 365

TTC AAA AGA TTG ATT GAA GGA AAC ACA ACA TTT CTG AAC TGG ACT CCT  1152
Phe Lys Arg Leu Ile Glu Gly Asn Thr Thr Phe Leu Asn Trp Thr Pro
    370                 375                 380

TAC GGT GGT ATG ATG TCG AAA ATC CCT GAA TCT GCG ATC CCA TTT CCG  1200
Tyr Gly Gly Met Met Ser Lys Ile Pro Glu Ser Ala Ile Pro Phe Pro
385                 390                 395                 400

CAT AGA AAC GGA ACC CTC TTC AAG ATT CTC TAT TAC GCG AAC TGG CTA  1248
His Arg Asn Gly Thr Leu Phe Lys Ile Leu Tyr Tyr Ala Asn Trp Leu
                405                 410                 415

GAG AAT GAC AAG ACA TCG AGT AGA AAA ATC AAC TGG ATC AAA GAG ATA  1296
Glu Asn Asp Lys Thr Ser Ser Arg Lys Ile Asn Trp Ile Lys Glu Ile
            420                 425                 430

TAC AAT TAC ATG GCG CCT TAT GTC TCA AGC AAT CCA AGA CAA GCA TAT  1344
Tyr Asn Tyr Met Ala Pro Tyr Val Ser Ser Asn Pro Arg Gln Ala Tyr
        435                 440                 445

GTG AAC TAC AGA GAT CTA GAC TTC GGA CAG AAC AAG AAC AAC GCA AAG  1392
Val Asn Tyr Arg Asp Leu Asp Phe Gly Gln Asn Lys Asn Asn Ala Lys
    450                 455                 460
```

```
    GTT AAC TTC ATT GAA GCT AAA ATC TGG GGA CCT AAG TAC TTC AAA GGC  1440
    Val Asn Phe Ile Glu Ala Lys Ile Trp Gly Pro Lys Tyr Phe Lys Gly
    465                 470                 475                 480

 5  AAT TTT GAC AGA TTG GTG AAG ATT AAA ACC AAG GTT GAT CCA GAG AAC  1488
    Asn Phe Asp Arg Leu Val Lys Ile Lys Thr Lys Val Asp Pro Glu Asn
                    485                 490                 495

    TTC TTC AGG CAC GAG CAG AGT ATC CCA CCT ATG CCC TAC TAG          1530
10  Phe Phe Arg His Glu Gln Ser Ile Pro Pro Met Pro Tyr
                500                 505
```

(2) INFORMATION FOR SEQ ID NO: 75:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 509 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

```
    Ser Ile Gln Asp Gln Phe Ile Asn Cys Val Lys Arg Asn Thr His Val
25   1               5                  10                  15

    Ser Phe Pro Leu Glu Lys Thr Leu Phe Thr Pro Ala Lys Asn Val Ser
                20                  25                  30

30  Leu Phe Asn Gln Val Leu Glu Ser Thr Ala Gln Asn Leu Gln Phe Leu
            35                  40                  45

    Ala Lys Ser Met Pro Lys Pro Gly Phe Ile Phe Arg Pro Ile His Gln
        50                  55                  60
35
    Ser Gln Val Gln Ala Ser Ile Ile Cys Ser Lys Lys Leu Gly Ile His
    65                  70                  75                  80

    Phe Arg Val Arg Ser Gly Gly His Asp Phe Glu Ala Leu Ser Tyr Val
40                  85                  90                  95

    Ser Arg Ile Glu Lys Pro Phe Ile Leu Leu Asp Leu Ser Lys Leu Lys
                100                 105                 110

45  Gln Ile Asn Val Asp Ile Glu Ser Asn Ser Ala Trp Val Gln Pro Gly
            115                 120                 125

    Ala Thr Leu Gly Glu Leu Tyr Tyr Arg Ile Ala Glu Lys Ser Lys Ile
        130                 135                 140
50
    His Gly Phe Pro Ala Gly Leu Cys Thr Ser Val Gly Ile Gly Gly Tyr
    145                 150                 155                 160

    Met Thr Gly Gly Gly Tyr Gly Thr Leu Met Arg Lys Tyr Gly Leu Ala
55                  165                 170                 175
```

Gly Asp Asn Val Leu Asp Val Lys Met Val Asp Ala Asn Gly Lys Leu
180 185 190

Leu Asp Arg Ala Ala Met Gly Glu Asp Leu Phe Trp Ala Ile Arg Gly
195 200 205

Gly Gly Gly Ala Ser Phe Gly Ile Val Leu Ala Trp Lys Ile Lys Leu
210 215 220

Val Pro Val Pro Lys Thr Val Thr Val Phe Thr Val Thr Lys Thr Leu
225 230 235 240

Glu Gln Asp Ala Arg Leu Lys Thr Ile Ser Lys Trp Gln Gln Ile Ser
245 250 255

Ser Lys Ile Ile Glu Glu Ile His Ile Arg Val Val Leu Arg Ala Ala
260 265 270

Gly Asn Asp Gly Asn Lys Thr Val Thr Met Thr Tyr Leu Gly Gln Phe
275 280 285

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290 295 300

Glu Leu Gly Leu Thr Gln Lys Asp Cys Thr Glu Met Ser Trp Ile Glu
305 310 315 320

Ala Ala Leu Phe His Gly Gly Phe Pro Thr Gly Ser Pro Ile Glu Ile
325 330 335

Leu Leu Gln Leu Lys Ser Pro Leu Gly Lys Asp Tyr Phe Lys Ala Thr
340 345 350

Ser Asp Phe Val Lys Glu Pro Ile Pro Val Ile Gly Phe Lys Gly Ile
355 360 365

Phe Lys Arg Leu Ile Glu Gly Asn Thr Thr Phe Leu Asn Trp Thr Pro
370 375 380

Tyr Gly Gly Met Met Ser Lys Ile Pro Glu Ser Ala Ile Pro Phe Pro
385 390 395 400

His Arg Asn Gly Thr Leu Phe Lys Ile Leu Tyr Tyr Ala Asn Trp Leu
405 410 415

Glu Asn Asp Lys Thr Ser Ser Arg Lys Ile Asn Trp Ile Lys Glu Ile
420 425 430

Tyr Asn Tyr Met Ala Pro Tyr Val Ser Ser Asn Pro Arg Gln Ala Tyr
435 440 445

Val Asn Tyr Arg Asp Leu Asp Phe Gly Gln Asn Lys Asn Asn Ala Lys
450 455 460

Val Asn Phe Ile Glu Ala Lys Ile Trp Gly Pro Lys Tyr Phe Lys Gly
465 470 475 480

Asn Phe Asp Arg Leu Val Lys Ile Lys Thr Lys Val Asp Pro Glu Asn
485 490 495

Phe Phe Arg His Glu Gln Ser Ile Pro Pro Met Pro Tyr
500 505

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Mogen International N.V.
Einsteinweg 97
2333 CB LEIDEN
Nederland

name and address of depositor

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
E. coli DH5 alpha strain / the plasmid pMOG800	CBS 414.93

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[X] a scientific description

[ ] a proposed taxonomic designation

(mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary accepts the microorganism identified under I above, which was received by it on Thursday, 12 August 1993 *(date of the original deposit)*[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on not applicable *(date of the original deposit)* and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on not applicable *(date of receipt of request for conversion)*

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Centraalbureau voor Schimmelcultures

Address: Oosterstraat 1
P.O. Box 273
3740 AG BAARN
The Netherlands

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

drs F.M. van Asma

Date: Friday, 13 August 1993

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

CBS/9107

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Mogen International N.V.
Einsteinweg 97
2333 CB LEIDEN
Nederland

name and address of the party to whom the viability statement is issued

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITORY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Mogen International N.V. Address: Einsteinweg 97 2333 CB LEIDEN Nederland	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: CBS 414.93 Date of the deposit or of the transfer:[1] Thursday, 12 August 1993

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on Friday, 13 August 1993 [2]. On that date, the said microorganism was

[X][3] viable

[ ][3] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY HAS BEEN PERFORMED [4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Centraalbureau voor Schimmelcultures

Address: Oosterstraat 1
P.O. Box 273
3740 AG BAARN
The Netherlands

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

drs F.M. van Asma

Date: Friday, 13 August 1993

[4] Fill in if the information has been requested and if the results of the test were negative.

CLAIMS

1. An isolated protein which has antifungal activity, preferably anti-Oomycete activity, more preferably anti-*Phytophthora* and/or anti-*Pythium* activity and which is obtainable from a plant source, encoded by the nucleotide sequence as shown in SEQ ID NO: 15, SEQ ID NO: 18, SEQ ID NO: 57, SEQ ID NO:70, SEQ ID NO:72 or SEQ ID NO:74 or parts or muteins therefrom.

2. An isolated protein according to claim 1, characterised in that it is obtainable from sunflower or lettuce plants.

3. An isolated protein having carbohydrate oxidase activity, characterised in that it has antifungal activity, preferably anti-Oomycete activity and more preferably anti-*Phytophthora* and/or anti-*Pythium* activity.

4. A carbohydrate oxidase, characterized in that it has an amino acid sequence according to SEQ ID NO: 16, SEQ ID NO: 19, SEQ ID NO: 58, SEQ ID NO: 71, SEQ ID NO:73 or SEQ ID NO: 75 or parts or muteins therefrom.

5. An isolated protein, characterised in that it comprises one or more of the peptides selected from the group consisting of:

(a) amino acids 1 to 25 of SEQ ID NO: 1,
(b) amino acids 1 to 25 of SEQ ID NO: 2,
(c) amino acids 1 to 118 of SEQ ID NO: 6,
(d) amino acids 1 to 529 of SEQ ID NO: 16, or a part of said sequence having antifungal activity,
(e) amino acids 1 to 529 of SEQ ID NO: 20, or a part of said sequence having antifungal activity,
(f) amino acids 1 to 21 of SEQ ID NO: 49
(g) amino acids 1 to 24 of SEQ ID NO: 50
(h) amino acids 1 to 14 of SEQ ID NO: 51
(i) amino acids 1 to 540 of SEQ ID NO: 58, or a part of said sequence having antifungal activity,
(j) amino acids 1 to 508 of SEQ ID NO: 71, or a part of said sequence having antifungal activity,
(j) amino acids 1 to 508 of SEQ ID NO: 73, or a part of said sequence

having antifungal activity,
(j) amino acids 1 to 509 of SEQ ID NO: 75, or a part of said sequence having antifungal activity,
as well as muteins thereof which have antifungal activity.

6. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 15, or by part of said open reading frame.

7. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 57, or by part of said open reading frame.

8. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 70, or by part of said open reading frame.

9. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 72, or by part of said open reading frame.

10. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 74, or by part of said open reading frame.

11. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by the open reading frame represented by SEQ ID NO: 19, or by part of said open reading frame.

12. An antifungal protein comprising an amino acid sequence characterised in that it is capable of being encoded by one of the open reading frames represented by SEQ ID NO's: 21 - 48.

13. An isolated DNA sequence comprising an open reading frame capable of encoding a protein according to any of the claims 1 to 12, and DNA capable of hybridising therewith under stringent conditions.

14. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 5.

15. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 15.

16. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 19.

17. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 21-48.

18. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 57.

19. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 70.

20. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 72.

21. An isolated DNA sequence according to claim 13, characterised in that it comprises the nucleotide sequence depicted in SEQ ID NO: 74.

22. A chimeric DNA sequence comprising a DNA sequence according to any of claims 13 to 21.

23. A chimeric DNA sequence according to claim 22, further comprising a transcriptional initiation region and, optionally, a transcriptional termination region, so linked to said open reading frame as to enable the chimeric DNA to be transcribed in a living host cell when present therein, thereby producing RNA which comprises said open reading frame.

24. A chimeric DNA sequence according to claim 23, wherein the RNA comprising said open reading frame is capable of being translated into protein in said host cell, when present therein, thereby producing said protein.

25. A chimeric DNA sequence according to any one of claims 22 to 24 which is a replicon, preferably pMOG1144 or pMOG1180.

26. A chimeric DNA sequence according to claim 25 which is a vector.

27. A vector according to claim 26, which is a binary vector, preferably pMOG1144 or pMOG1180.

28. A host cell comprising a replicon according to claim 25 and which is capable of maintaining said replicon once present therein.

29. A host cell comprising a vector according to claim 26 or 27 and which is capable of maintaining said vector once present therein.

30. A host cell stably incorporating in its genome a chimeric DNA sequence according to claim 22 or 23.

31. A host cell according to claim 30 which is a plant cell, said vector being a non-integrative viral vector.

32. A host cell according to claim 30 which is a plant cell.

33. A plant or a plant part comprising at least one plant cell according to claim 31 or 32.

34. A plant or a plant part consisting essentially of plant cells according to claim 32.

35. A plant according to claim 34, characterised in that said chimeric DNA is expressed in at least a number of the plant's cells causing the said antifungal protein to be produced therein.

36. A method for the production of a protein with antifungal activity, preferably anti-anti-Oomycete activity, more preferably anti-*Phytophthora* activity and/or anti-*Pythium* activity, characterised in that a host cell according to claim 29 to 32 is grown under conditions allowing the said protein to be produced by said host cells.

37. A method according to claim 36, further comprising the step of recovering the protein from the host cells.

38. Use of a protein according to any one of claims 1 to 12 for retarding fungal growth, preferably Oomycete growth and more preferably the growth of *Phytophthora sp.* and/or *Pythium sp.*

39. A plant-derived protein having a molecular weight of 55-65 kD, characterized in that it has carbohydrate oxidising activity.

40. A protein according to claim 39, characterized in that it is a hexose oxidase.

41. A protein according to claim 40, characterized in that it is obtainable from sunflower or lettuce plants.

42. A method of retarding the growth of the a fungus, preferably an Oomycete, more preferably *Phytophthora* or *Pythium* on plant leaves, characterised in that the plant is treated with a protein produced from a host cell according to claim 29 or 30, or from a cell of a plant according to claim 35.

43. A method for obtaining plants with reduced susceptibility to fungi, preferbly Oomycetes, more preferably *Phytophthora* or *Pythium*, comprising the steps of

(a) introducing into ancestor cells which are susceptible of regeneration into a whole plant,

- a chimeric DNA sequence comprising an open reading frame capable of encoding a protein according to any of claims 1-12, said open reading frame being operatively linked to a transcriptional and translational region and, optionally, a transcriptional termination

region, allowing the said protein to be produced in a plant cell that is susceptible to infection by said fungus and

- a chimeric DNA sequence capable of encoding a plant selectable marker allowing selection of transformed ancestor cells when said selectable marker is present therein, and

(b) regenerating said ancestor cells into a plant under conditions favouring ancestor cells which have the said selectable marker, and

(c) identifying a plant which produces a protein according to claim 1-7, thereby reducing the susceptibility of said plant to infection by said fungus.

44. The method according to claim 43, characterised in that step (a) is performed using an *Agrobacterium tumefaciens* strain capable of T-DNA transfer to plant cells and which harbours a binary vector, and wherein step (b) is performed in the presence of an antibiotic favouring cells which have a neomycin phosphotransferase.

45. An antifungal composition comprising a protein according to any one of claims 1 to 12, and a suitable carrier.

46. An antibody capable of recognising a protein according to any one of claims 1 to 12.

47. A nucleic acid sequence obtainable from a gene encoding a protein according to any one of claims 1 to 12, having tissue-specific and/or developmental specific transcriptional regulatory activity in a plant.

48. A nucleic acid sequence according to claim 47, which is obtainable from the region upstream of the translational initiation site of said gene.

49. A nucleic acid sequence according to claim 48, which has at least 1000 nucleotides of said region upstream of the translational initiation site of said gene.

50. Use of a nucleic acid sequence according to any one of claims 47 to 49 for making a plant expressible gene construct.

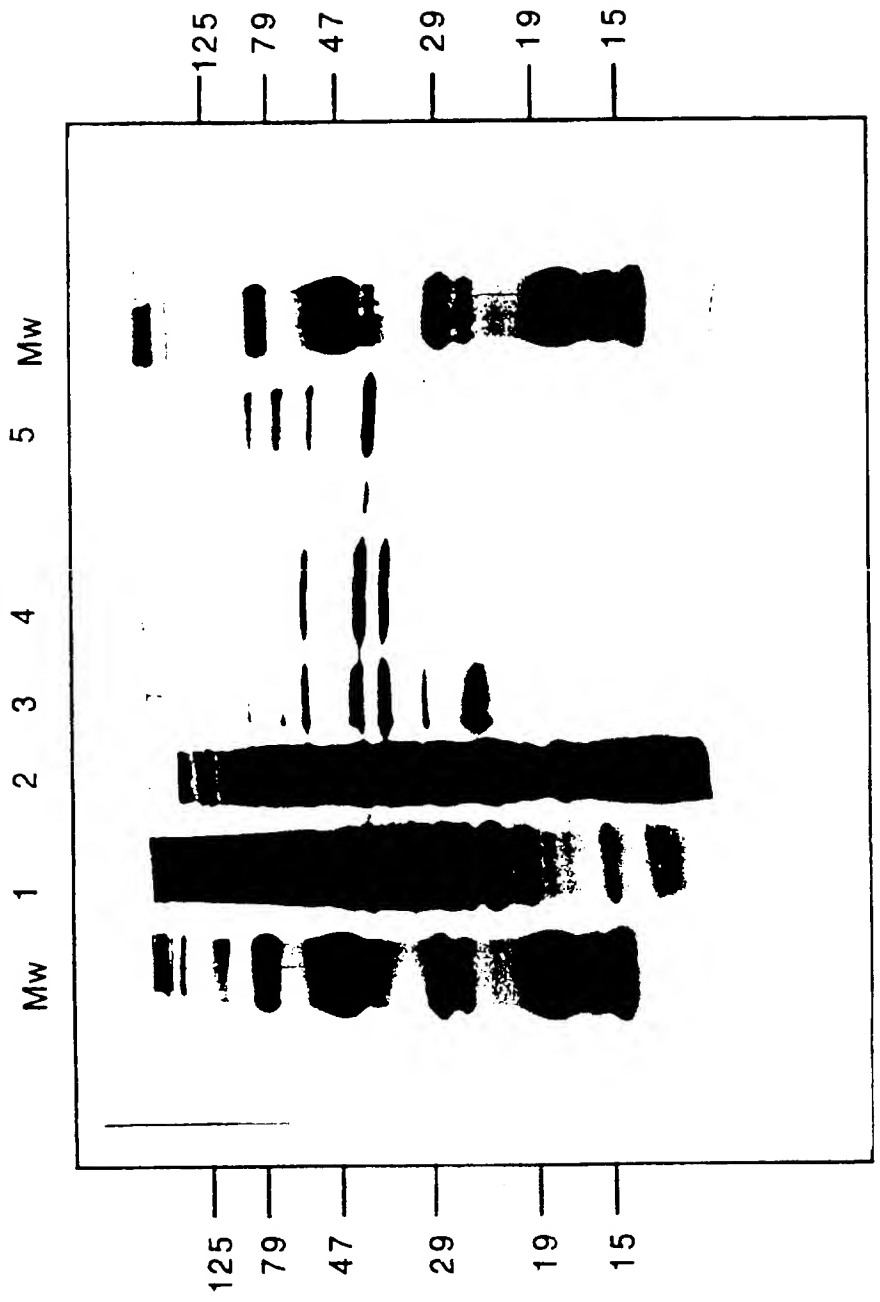
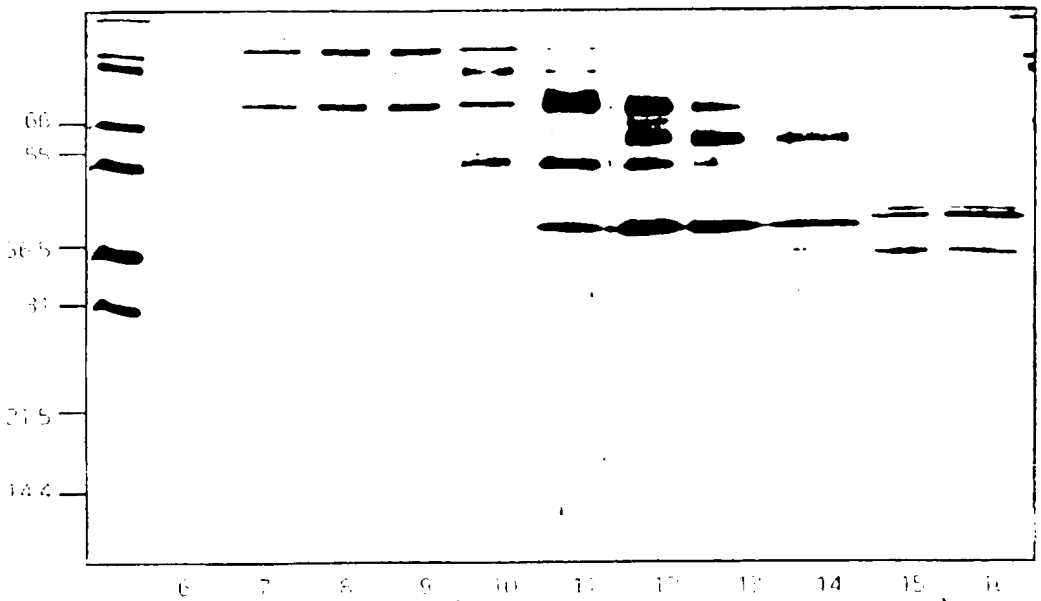
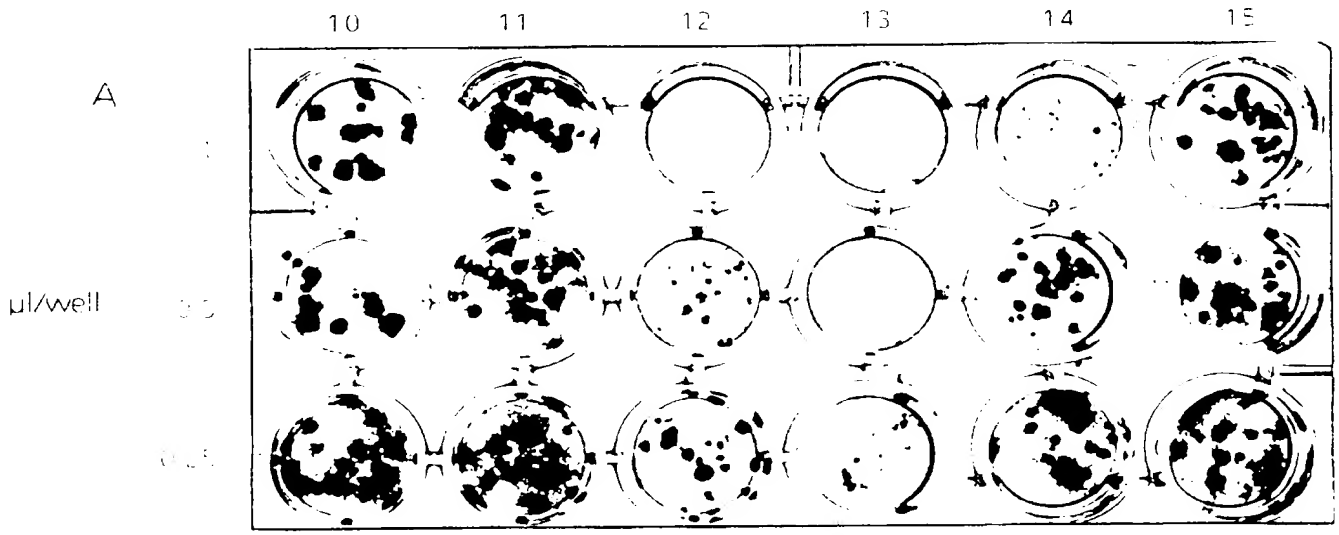


Fig. 1





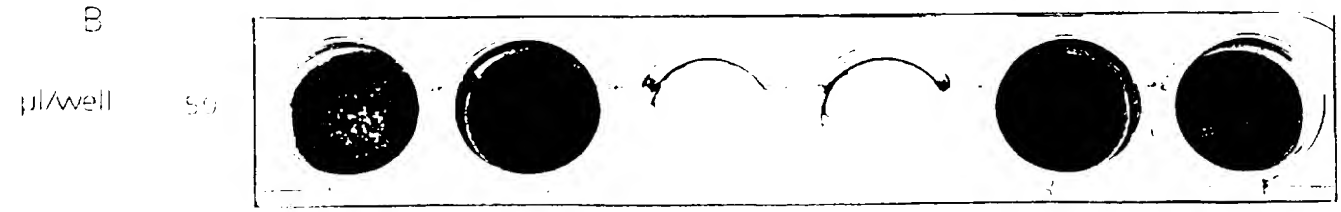


Fig. 2

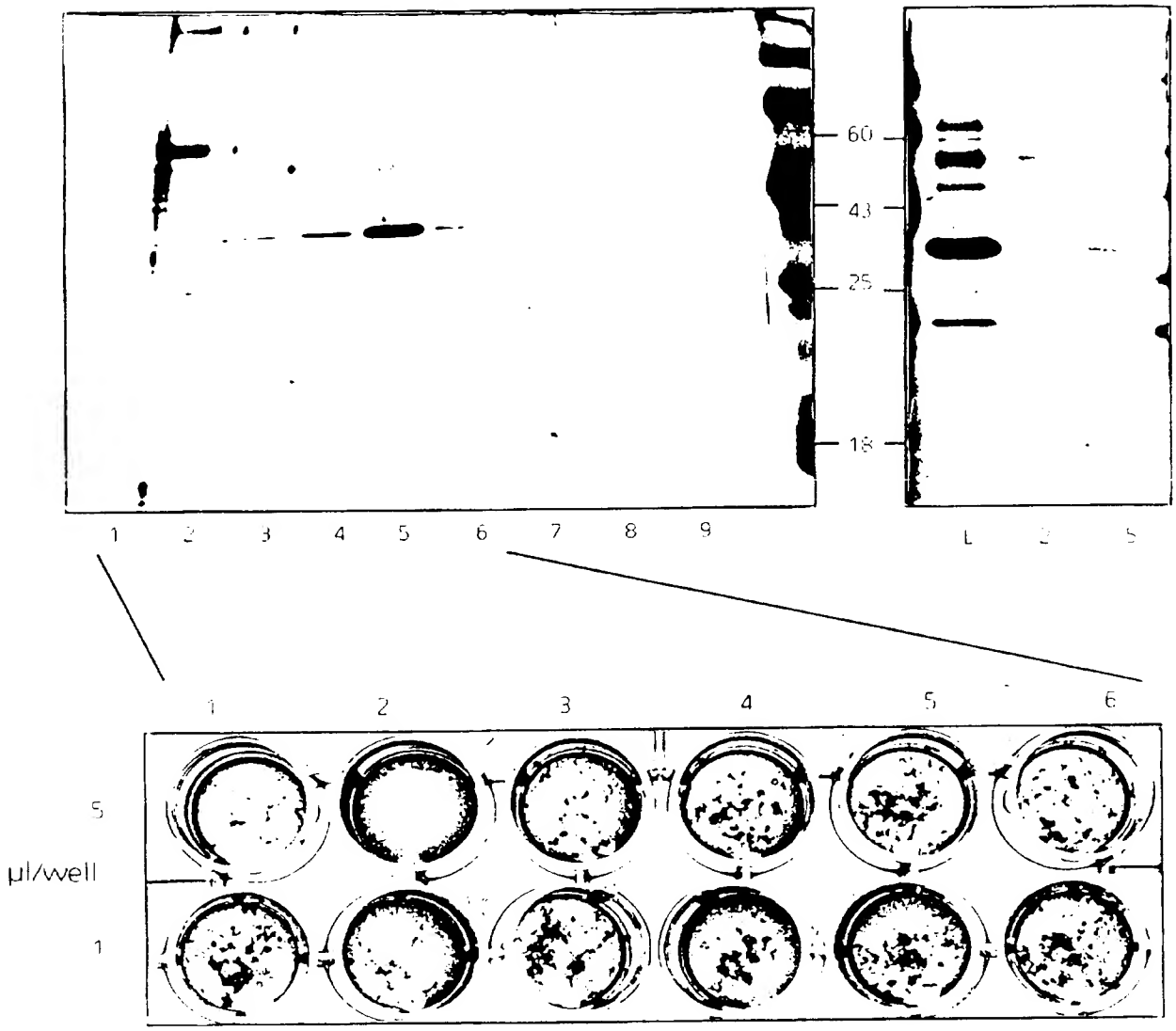
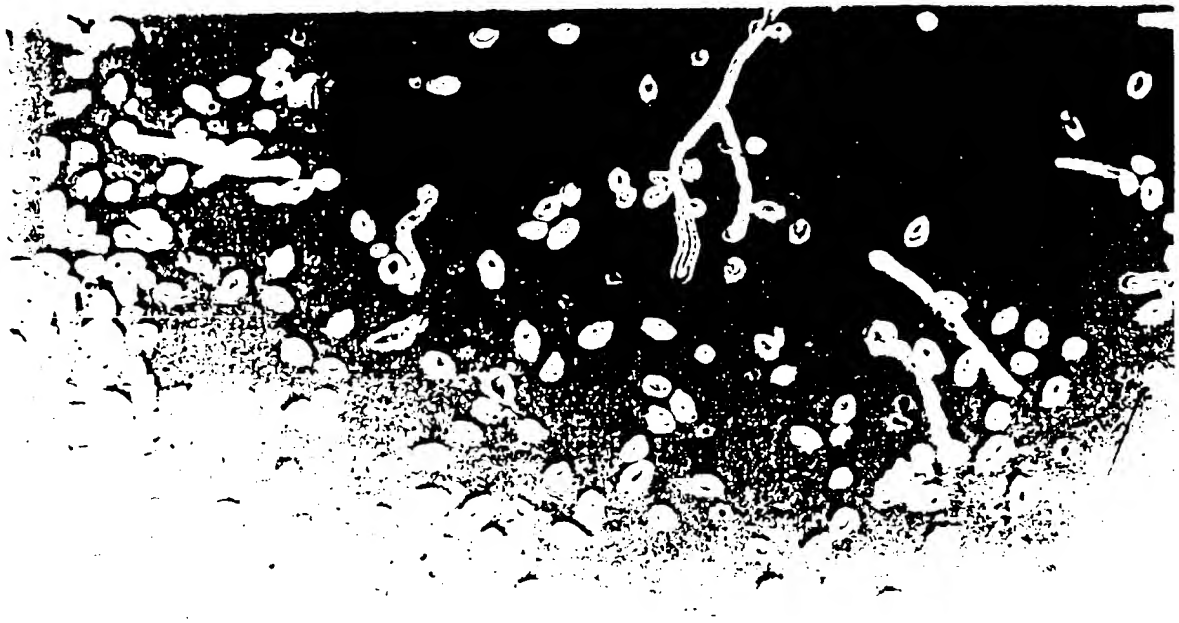


Fig. 3



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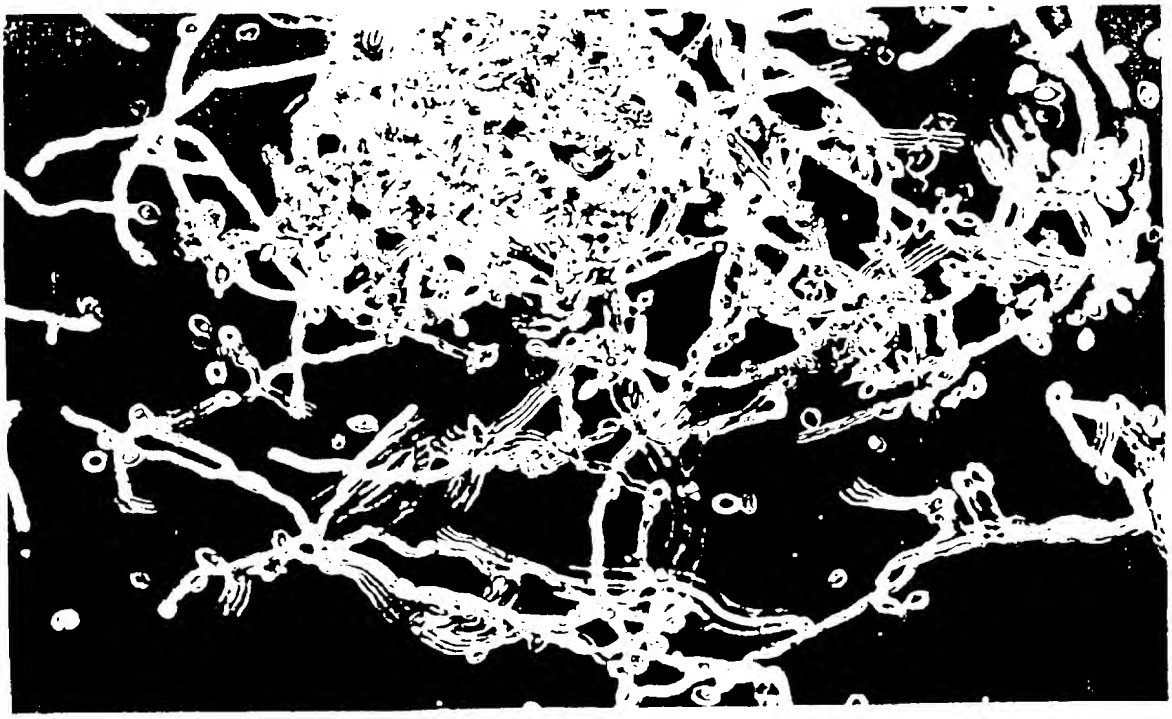

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Fig. 4

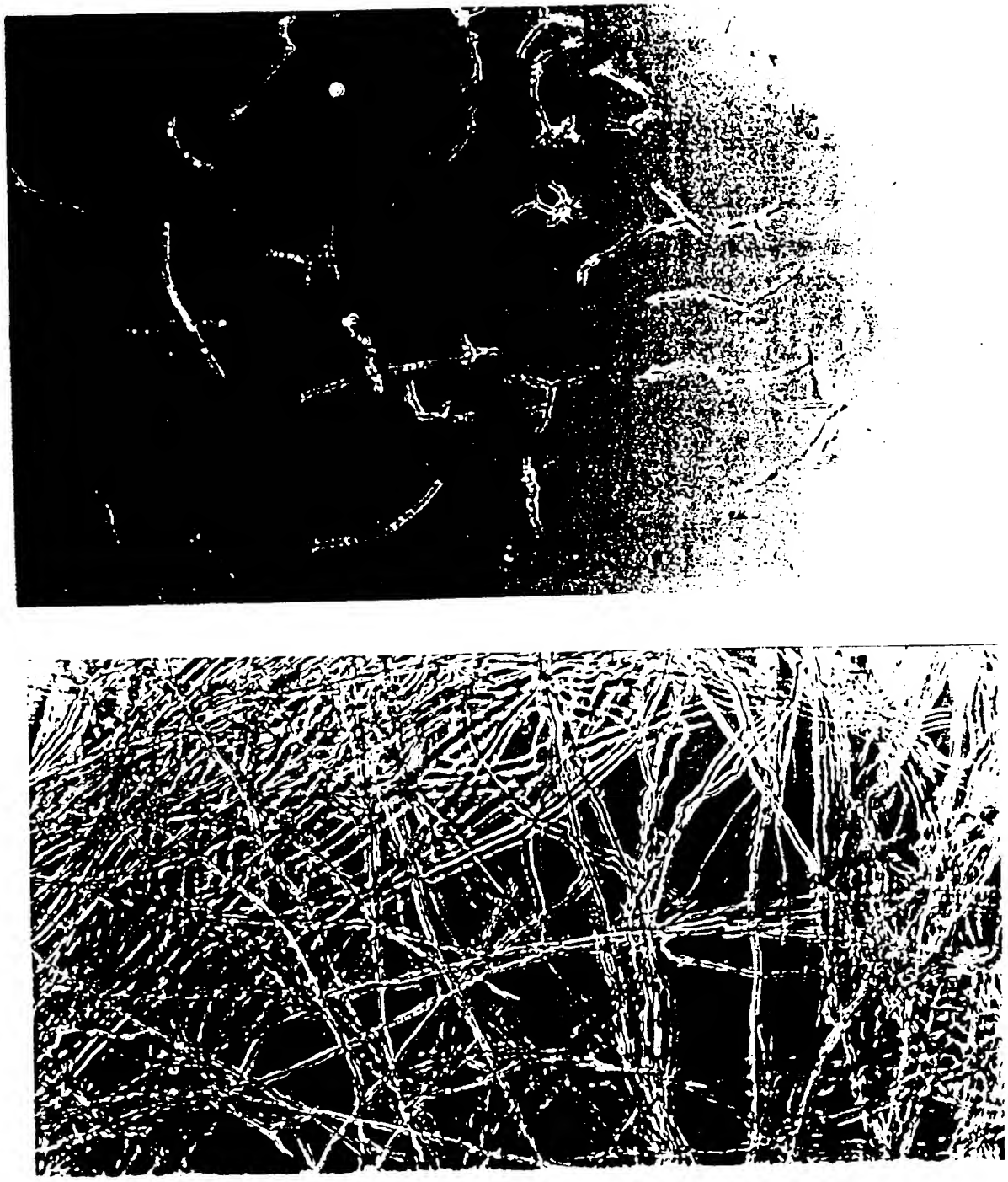
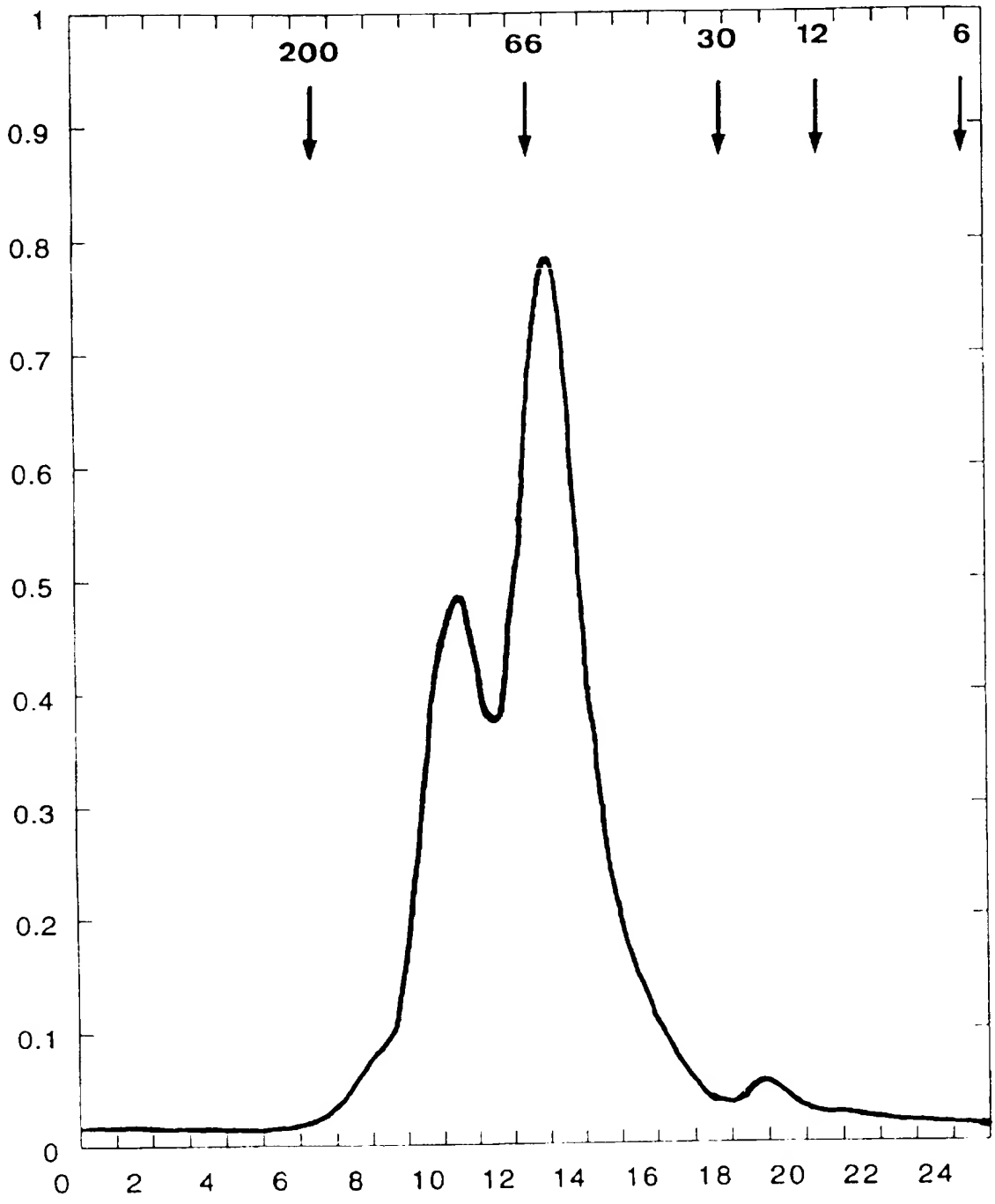


Fig. 5

6 A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6
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0
0 2 4 6 8 10 12 14 16 18 20 22 24

Fig. 6-1

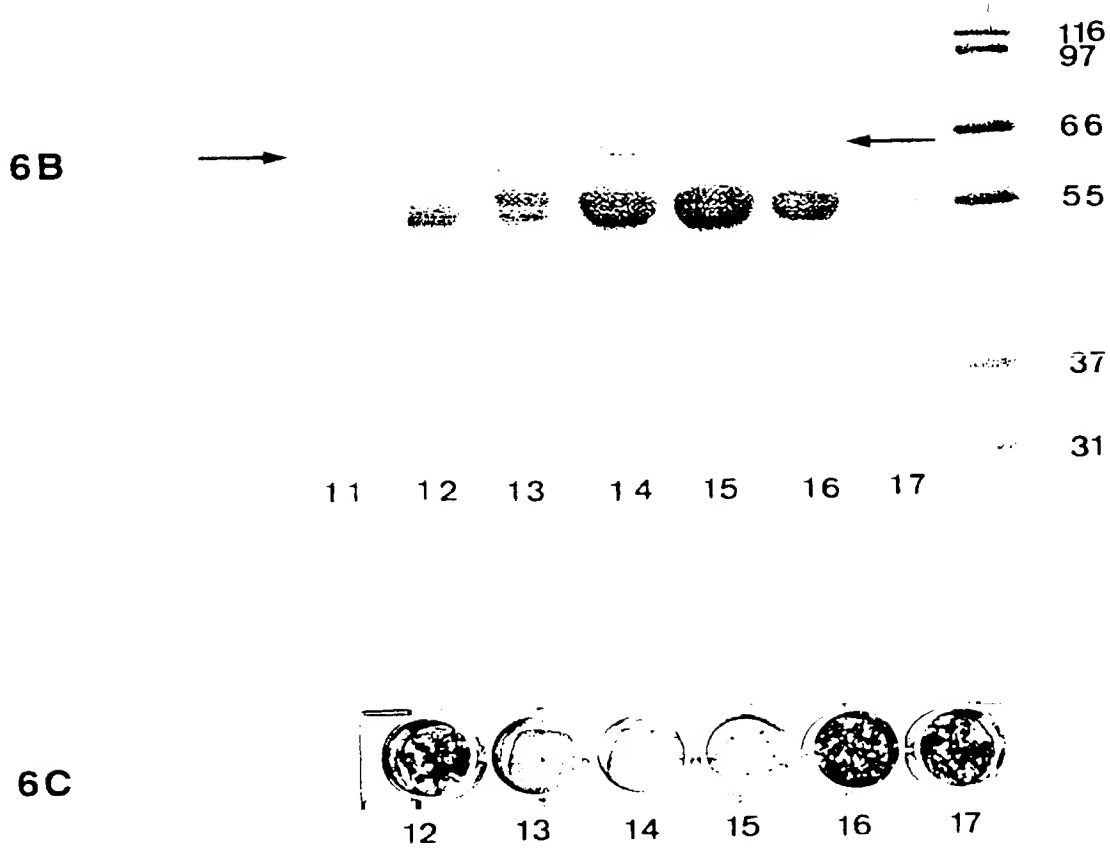


Fig. 6-2

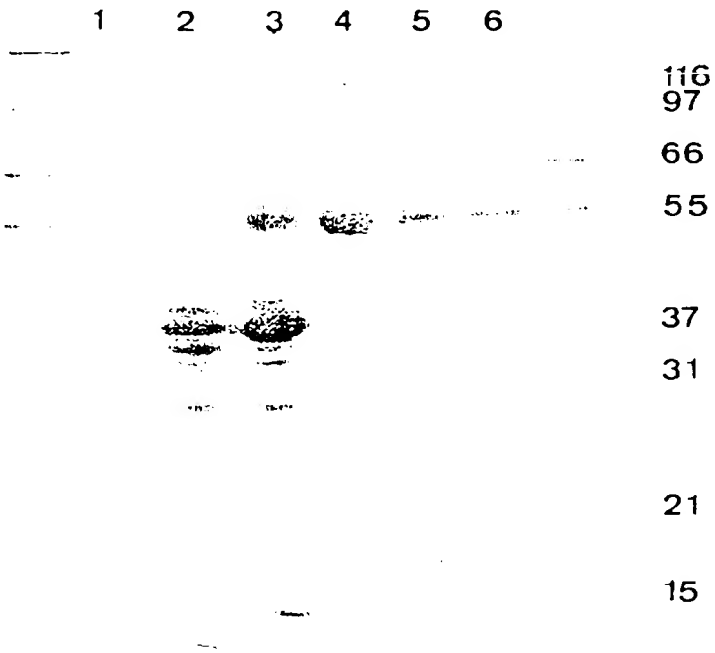
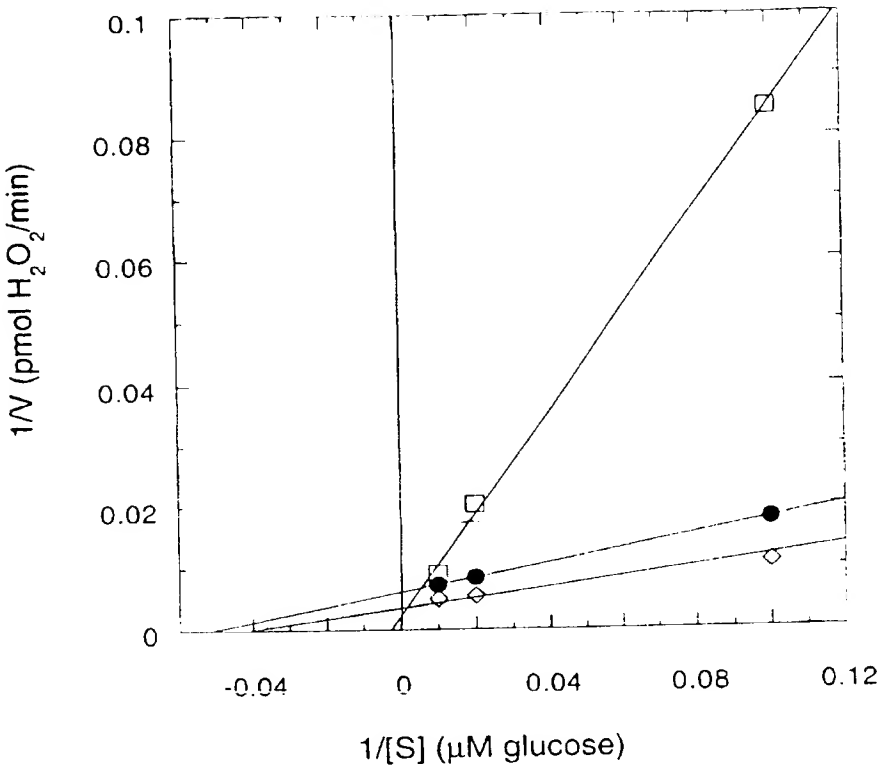


Fig. 7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0.04
0.02
0
1/V (pmol H_2O_2/min)
-0.04
0
0.04
0.08
0.12
1/[S] (µM glucose)

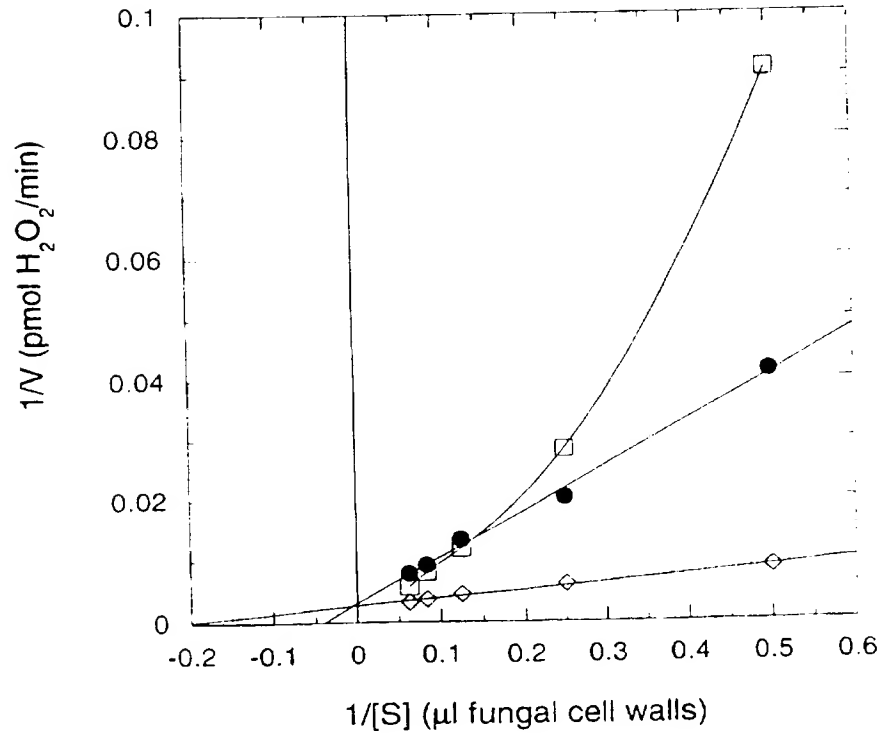
0.1
0.08
0.06
0.04
0.02
0
1/V (pmol H_2O_2/min)
-0.2
-0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
1/[S] (µl fungal cell walls)

Fig. 8

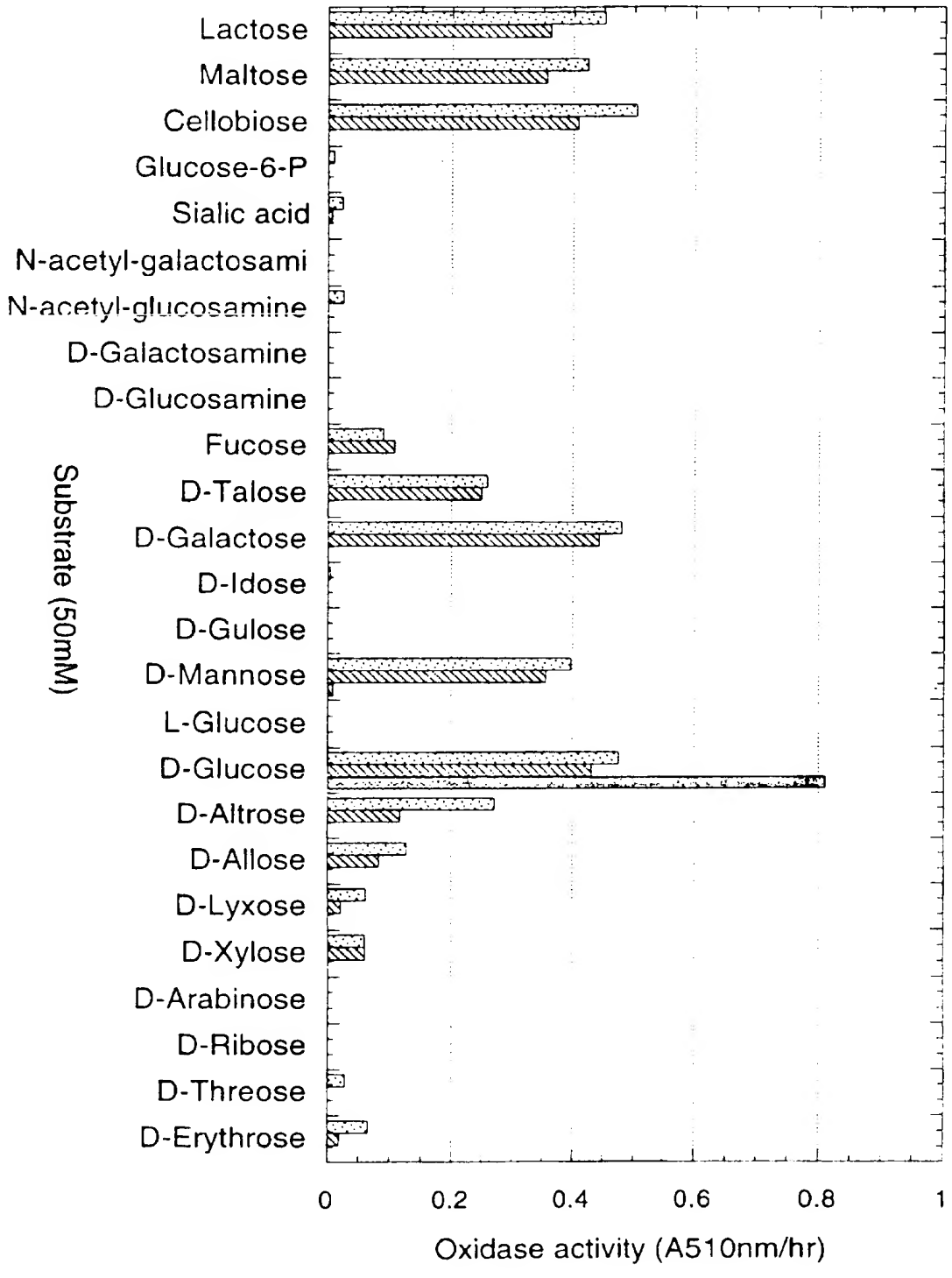

Fig. 9

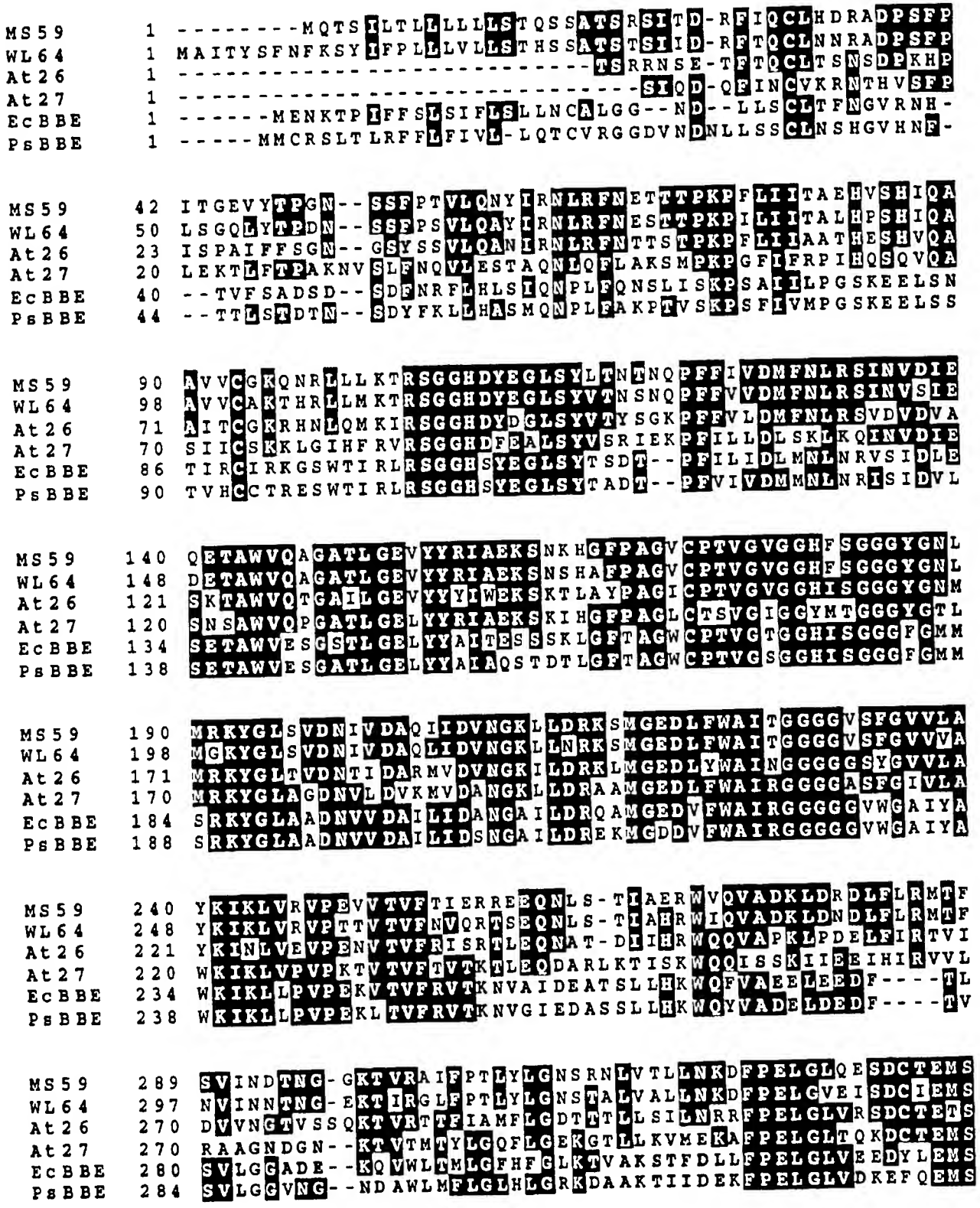
MS59 1 --------MQTSILTLLLLLLSTQSSATSRSITD-RFIQCLHDRADPSFP
WL64 1 MAITYSFNFKSYIFPLLLVLLSTHSSATSTSIID-RFTQCLNNRADPSFP
At26 1 ----------TSRRNSE-TFTQCLTSNSDPKHP
At27 1 ----------SIQD-QFINCVKRNTHVSFP
EcBBE 1 ------MENKTPIFFSLSIFLSLLNCALGG--ND--LLSCLTFNGVRNH-
PsBBE 1 -----MMCRSLTLRFFLFIVL-LQTCVRGGDVNDNLLSSCLNSHGVHNF-
MS59 42 ITGEVYTPGN--SSFPTVLQNYIRNLRFNETTTPKPFLIITAEHVSHIQA
WL64 50 LSGQLYTPDN--SSFPSVLQAYIRNLRFNESTTPKPILIITALHPSHIQA
At26 23 ISPAIFFSGN--GSYSSVLQANIRNLRFNTTSTPKPFLIIAATHESHVQA
At27 20 LEKTLFTPAKNVSLFNQVLESTAQNLQFLAKSMPKPGFIFRPIHQSQVQA
EcBBE 40 --TVFSADSD--SDFNRFLHLSIQNPLFQNSLISKPSAIILPGSKEELSN
PsBBE 44 --TTLSTDTN--SDYFKLLHASMQNPLFAKPTVSKPSFIVMPGSKEELSS
MS59 90 AVVCGKQNRLLLKTRSGGHDYEGLSYLTNTNQPFFIVDMFNLRSINVDIE
WL64 98 AVVCAKTHRLLMKTRSGGHDYEGLSYVTNSNQPFFVVDMFNLRSINVSIE
At26 71 AITCGKRHNLQMKIRSGGHDYDGLSYVTYSGKPFFVLDMFNLRSVDVDVA
At27 70 SIICSKKLGIHFRVRSGGHDFEALSYVSRIEKPFILLDLSKLKQINVDIE
EcBBE 86 TIRCIRKGSWTIRLRSGGHSYEGLSYTSDT--PFILIDLMNLNRVSIDLE
PsBBE 90 TVHCCTRESWTIRLRSGGHSYEGLSYTADT--PFVIVDMMNLNRISIDVL
MS59 140 QETAWVQAGATLGEVYYRIAEKSNKHGFPAGVCPTVGVGGHFSGGGYGNL
WL64 148 DETAWVQAGATLGEVYYRIAEKSNSHAFPAGVCPTVGVGGHFSGGGYGNL
At26 121 SKTAWVQTGAILGEVYYYIWEKSKTLAYPAGICPTVGVGGHISGGGYGNM
At27 120 SNSAWVQPGATLGELYYRIAEKSKIHGFPAGLCTSVGIGGYMTGGGYGTL
EcBBE 134 SETAWVESGSTLGELYYAITESSSKLGFTAGWCPTVGTGGHISGGGFGMM
PsBBE 138 SETAWVESGATLGELYYAIAQSTDTLGFTAGWCPTVGSGGHISGGGFGMM
MS59 190 MRKYGLSVDNIVDAQIIDVNGKLLDRKSMGEDLFWAITGGGGVSFGVVLA
WL64 198 MGKYGLSVDNIVDAQLIDVNGKLLNRKSMGEDLFWAITGGGGVSFGVVVA
At26 171 MRKYGLTVDNTIDARMVDVNGKILDRKLMGEDLYWAINGGGGGSYGVVLA
At27 170 MRKYGLAGDNVLDVKMVDANGKLLDRAAMGEDLFWAIRGGGGASFGIVLA
EcBBE 184 SRKYGLAADNVVDAILIDANGAILDRQAMGEDVFWAIRGGGGGVWGAIYA
PsBBE 188 SRKYGLAADNVVDAILIDSNGAILDREKMGDDVFWAIRGGGGGVWGAIYA
MS59 240 YKIKLVRVPEVVTVFTIERREEQNLS-TIAERWVQVADKLDRDLFLRMTF
WL64 248 YKIKLVRVPTTVTVFNVQRTSEQNLS-TIAHRWIQVADKLDNDLFLRMTF
At26 221 YKINLVEVPENVTVFRISRTLEQNAT-DIIHRWQQVAPKLPDELFIRTVI
At27 220 WKIKLVPVPKTVTVFTVTKTLEQDARLKTISKWQQISSKIIEEIHIRVVL
EcBBE 234 WKIKLLPVPEKVTVFRVTKNVAIDEATSLLHKWQFVAEELEEDF----TL
PsBBE 238 WKIKLLPVPEKLTVFRVTKNVGIEDASSLLHKWQYVADELDEDF----TV
MS59 289 SVINDTNG-GKTVRAIFPTLYLGNSRNLVTLLNKDFPELGLQESDCTEMS
WL64 297 NVINNTNG-EKTIRGLFPTLYLGNSTALVALLNKDFPELGVEISDCIEMS
At26 270 DVVNGTVSSQKTVRTTFIAMFLGDTTTLLSILNRRFPELGLVRSDCTETS
At27 270 RAAGNDGN--KTVTMTYLGQFLGEKGTLLKVMEKAFPELGLTQKDCTEMS
EcBBE 280 SVLGGADE--KQVWLTMLGFHFGLKTVAKSTFDLLFPELGLVEEDYLEMS
PsBBE 284 SVLGGVNG--NDAWLMFLGLHLGRKDAAKTIIDEKFPELGLVDKEFQEMS

Fig. 10-1

```
MS59    338  WVESVLYYTGFPSGTPTTALLSRTP-QRLNPFKIKSDYVQNPISKRQFEF
WL64    346  WIESVLFYTNFPIGTPTTALLSRTP-QRLNPFKIKSDYVKNTISKQGFES
At26    320  WIQSVLFWTNIQVGSSETLLLQRN--QPVNYLKRKSDYVREPISRTGLES
At27    318  WIEAALFHGGFPTGSPIEILLQLKSPLGKDYFKATSDFVKEPIPVIGFKG
EcBBE   328  WGESFAYLAGLET---VSQLNNRFLKFDERAFKTKVDLTKEPLPSKAFYG
PsBBE   332  WGESMAFLSGLDT---ISELNNRFLKFDERAFKTKVDFTKVSVPLNVFRH

MS59      1  FIFERLKELENQMLAFNPYGGRMSEISEFAKPFPHRSGNIAKIQYEVNWE
WL64      1  SIFERMKELENQMLAFNPYGGRMSEISEFAKPFPHRSGNIAKIQYEVNWD
At26      1  SIWKKMIELEIPTMAFNPYGGEMGRISSTVTPFPYRAGNLWKIQYGANWR
At27      1  GIFKRLISGNTTFLNWTPYGGMMSKIPESAIPFPHRNGTLFKILYYANWL
EcBBE     1  GLLERLSKEPNGFIALNGFGGQMSKISSDFTPFPHRSGTRLMVEYIVAWN
PsBBE     1  HALEMLSEQPGGFIALNGFGGKMSEISTDFTPFPHRKGTKLMFEYIIAWN

MS59     51  DLSDEAENRYLNFTRLMYDYMTPFVSKNPRKAFLNYRDLDIG-INSHG--
WL64     51  ELGVEAANRYLNFTRVMYDYMTPFVSKNPREAFLNYRDLDIG-VNSHG--
At26     51  DET--LTDRYMELTRKLYQFMTPFVSKNPRQSFFNYRDVDLG-INSHNG-
At27     51  END-KTSSRKINWIKEIYNYMAPYVSSNPRQAYVNYRDLDFG-QNKNN--
EcBBE    51  QSEQKKKTEFLDWLEKVYEFMKPFVSKNPRLGYVNHIDLDLGGIDWGNKT
PsBBE    51  QDEESKIGEFSEWLAKFYDYLEPFVSKEPRVGYVNHIDLDIGGIDWRNKS

MS59     98  -RNAYTEGMV-YGHKYFKETNYKRLVSVKTKVDPDNFFRNEQSIPTLSS-
WL64     98  -KNAYGEGMV-YGHKYFKETNYKRLTMVKTRVDPSNFFRNEQSIPTLSSS
At26     97  KISSYVEGKR-YGKKYFAG-NFERLVKIKTRVDSGNFFRNEQSIPVLP--
At27     97  AKVNFIEAKI-WGPKYFKG-NFDRLVKIKTKVDPENFFRHEQSIPPMPY-
EcBBE   101  VVNNAIEISRSWGESYFLS-NYERLIRAKTLIDPNNVFNHPQSIPPMANF
PsBBE   101  STTNAVEIARNWGERYFSS-NYERLVKAKTLIDPNNVFNHPQSIPPMMKF

MS59        ----------------
WL64    146 WK----------------
At26        ----------------
At27        ----------------
EcBBE   150 D--YLEKTLGSDGGEVVI
PsBBE   150 EEIYMLKEL---------
```

Fig. 10-2

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(71) Applicant *(for all designated States except US)*: MOGEN INTERNATIONAL N.V. [NL/NL]; Einsteinweg 97, NL–2333 CB Leiden (NL).

(72) Inventors; and
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(74) Agent: VAN WEZENBEEK, Bart; Mogen International N.V., Einsteinweg 97, NL–2333 CB Leiden (NL).

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(57) Abstract

The present invention provides an isolated protein obtainable from a plant source which has antifungal activity, specifically anti–*Phytophthora* activity and/or anti–*Pythium* activity and a molecular weight of about 55–65 kDa as judged by SDS PAGE–electrophoresis, an isolated DNA sequence comprising an open reading frame capable of encoding a protein according to the invention, preferably characterised in that it comprises an open reading frame which is capable of encoding a protein depicted in SEQ ID NO. 16, SEQ ID NO. 57, SEQ ID NO. 70, SEQ ID NO. 72 or SEQ ID NO. 74 or muteins thereof, and DNA capable of hybridising therewith under stringent conditions. The invention further comprises plants incorporating chimeric DNA capable of encoding a protein according to the invention, and wherein the protein is expressed. Also shown is the carbohydrate and preferably hexose oxidating activity of said protein. Also methods are provided for combating fungi, especially *Phytophthora* and *Pythium* species, using a protein or a host cell capable of producing the protein.

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INTERNATIONAL SEARCH REPORT

Interna' il Application No
PCT/EP 97/04923

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N9/02 C12Q1/68 C07K16/40 C12N15/62
A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 14784 A (MONSANTO CO) 1 June 1995 cited in the application abstract, pages 1,2, page 3 , lines 29-35; page 13, 15-17,29 ---	3,38,43, 44
X	WU, G., ET AL.: "DISEASE RESISTANCE CONFERRED BY EXPRESSION OF A GENE ENCODING H2O2-GENERATING GLUCOSE OXIDASE IN TRANSGENIC POTATO PLANTS" THE PLANT CELL, vol. 7, October 1995, pages 1357-1368, XP002065951 see the whole document --- -/--	3,38,43, 44

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
26 May 1998	02-07-1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	HOLTORF, S

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 97/04923

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KRIVITZKY, M., ET AL.: "THE ARABIDOPSIS THALIANA TRANSCRIBED GENOME: THE GDR cDNA PROGRAM" EMBL SEQUENCE DATA LIBRARY, HEIDELBERG, GERMANY, XP002025476 ACCESSION NO. F19886 ---	13,19,20
X	WO 95 21924 A (COMMW SCIENT IND RES ORG ;MURRAY FIONA RUTH (AU); LLEWELLYN DANNY) 17 August 1995 pages 4-7,13,15,18-21; examples 3,4, ---	3,38,43, 44
X	KAY KWANG-AE KIM ET AL: "GLUCOSE OXIDASE AS THE ANTIFUNGAL PRINCIPLE OF TALARON FROM TALAROMYCES FLAVUS" CANADIAN JOURNAL OF MICROBIOLOGY, vol. 36, no. 11, November 1990, pages 760-764, XP000602906 see the whole document ---	3
A	DITTRICH, H. AND KUTCHAN, T.M.: "MOLECULAR CLONING , EXPRESSION , AND INDUCTION OF BERBERINE BRIDGE ENZYME, AN ENZYME ESSENTIAL TO THE FORMATION OF BENZOPHENANTHRIDINE ALKALOIDS IN THE RESPONSE OF PLANTS TO PATHOGENIC ATTACK" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 88, November 1991, pages 9969-9973, XP002025475 cited in the application see the whole document ---	1-50
A	WO 95 21929 A (MOGEN INT ;MELCHERS LEO SJOERD (NL); PONSTEIN ANNE SILENE (NL); KR) 17 August 1995 abstract; pages 1-3,4,5,9,; page 11, lines 7-12, page 11, line40 - page 12, line 8; page 15-16, example 13; claims -----	1-50

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INTERNATIONAL SEARCH REPORT

Inte. ..tional application No.
PCT/EP 97/04923

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

The numbering of the SEQIDs is misleading; this concerns the SEQIDs 18 in claim 1 and SEQID 19 in claim 4.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

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☐ The additional search fees were accompanied by the applicant's protest.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna al Application No
PCT/EP 97/04923

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